VOLUME 17 NUMBER 3

GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

MAY, 1932

Published Bimonthly at Menasha, Wisconsin
by the
BROOKLYN BOTANIC GARDEN
Brooklyn, New York, U. S. A.

GENETICS

A Periodical Record of Investigations Bearing on Heredity and Variation

EDITORIAL BOARD

DONALD F. JONES, MANAGING EDITOR
Connecticut Agricultural Experiment Station

WILLIAM E. CASTLE
Harvard University

ROLLINS A. EMERSON
Cornell University

EDWIN G. CONKLIN
Princeton University

HERBERT S. JENNINGS
Johns Hopkins University

CHARLES B. DAVENPORT
Carnegie Institution of Washington

THOMAS H. MORGAN
California Institute of Technology

BRADLEY M. DAVIS
University of Michigan

RAYMOND PEARL
Johns Hopkins University

EDWARD M. EAST
Harvard University

GEORGE H. SHULL
Princeton University

C. STUART GAGER, BUSINESS MANAGER

Genetics is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $6.00 net a year for complete volumes (January-November). Parts of volumes are to be had only at the single number rate. Foreign postage, 50 cents additional. Single copies, $1.25 each, postpaid.

Back volumes, as available, may be had at $7.00 each, plus postage. The Business Manager will supply information on request as to odd volumes and numbers and complete sets available.

Correspondence concerning editorial matters should be addressed to the EDITOR OF GENETICS, P. O. Box 1106, New Haven, Conn.

Business Correspondence including change of address, directions concerning reprints, and exchanges, should be addressed to GENETICS, Menasha, Wisconsin, U.S.A., or BROOKLYN BOTANIC GARDEN, 1000 Washington Ave., Brooklyn, N. Y., U.S.A.

Remittances should be made payable to GENETICS.

Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

SELF-STERILITY IN SHEPHERD'S PURSE*

HERBERT PARKES RILEY
Princeton University, Princeton, New Jersey

Received August 17, 1931

TABLE OF CONTENTS

	PAGE
INTRODUCTION	231
Self- and cross-sterility in plants	232
Theoretical considerations	233
Materials and methods	239
Observations	242
Self-sterility	242
Cross-sterility	242
Intra-sterile, inter-fertile groups	242
Backcrosses	247
Offspring of reciprocal crosses	248
Families from a common mother and different fathers	250
Crosses involving fathers of a common class, but different mothers	251
Relations between self-fertile and self-sterile plants	253
Review of the literature	253
Observations	255
Crosses between *Capsella grandiflora* and self-fertile species	255
The F_1 generation	256
The F_2 generation	257
The F_3 generation	258
Crosses among F_2 segregates	258
Pseudo-fertility	260
Review of the literature	260
Observations	262
Physiology	265
Review of the literature	265
Observations	267
DISCUSSION	268
SUMMARY	273
LITERATURE CITED	275
APPENDIX	277

INTRODUCTION

Discovered in plants as early as 1764, by KOELREUTER, and in animals by CASTLE in 1896, the phenomenon known generally as self-sterility has been critically studied chiefly during the last two decades; while there has been great progress in determining its nature and causes in several organisms, only a beginning has been made, and it will require the exhaustive

* The cost of the accompanying tables is paid by the GALTON AND MENDEL MEMORIAL FUND.

Second Printing 1966 / University of Texas Printing Division, Austin

study of many other self-sterile species before a general understanding of the subject will have been attained.

The terminology employed by various investigators in this field has not been consistent. The term "self-sterility" has largely been applied to the inability of an hermaphrodite with functional gametes to self-fertilize when there are no mechanical barriers which will prevent a meeting of the gametes of such an animal or which will prevent self-pollination in flowers. Some authors prefer "self-incompatibility," and BRIEGER (1930) has proposed "self-parasterility"; but while these terms are expressive, there seems no pressing need to abandon the term which has been in use far more generally and for a much longer time. There should be no confusion from the use of "self-sterility," since the prefix "self" qualifies the expression and differentiates it from any form of *true* sterility; the word "sterility" alone is adequate for the latter.

Seed formation after a self- or cross-pollination will not occur if either gamete is impotent or if embryo abortion takes place after zygote formation. Dichogamy or structural dimorphism or trimorphism will also prevent self-fertilization and certain combinations of crosses. These phenomena, however, are different from self-sterility and they should not be confused with it, even though the end result in all these cases is the same, a failure to produce seeds. It is with self-sterility, or physiological incompatibility, that this paper is concerned, but the other phenomena which produce similar effects must also be considered so that they may not bring in complicating factors.

SELF- AND CROSS-STERILITY IN PLANTS

Self-sterility in plants is rather widespread and is sporadic for it occurs in many unrelated genera and is present often in but one species of a genus. To list here the many plants which show complete or partial self-sterility is unnecessary because of the recent reviews of BRIEGER (1930) and EAST (1929), and such a list would merely repeat much of their excellent works.

It is of great interest to note that in almost every instance of self-sterility, a condition of cross-sterility has been observed; and this association is so general that this type of cross-sterility must be considered a part of the self-sterility problem. Such cross-sterility often occurs among plants of the same species, and is in no way similar to that type of sterility exhibited by species hybrids. In the cross-sterility of self-sterile plants, the failure of seed production is due solely to physiological incompatibility, and any theory which attempts to account for self-sterility must consider this cross-sterility as well.

CORRENS (1928b) has reported an observation upon *Tolmiea Menziesii*, in which species he finds all plants self-sterile but reciprocally cross-fertile with one another, and fertile also with both parents. A condition similar to this has been reported in pears by FLORIN (1927). However, in most other cases of self-sterility which have been investigated, every plant is cross-sterile with some others, and all the plants of a species can be grouped together into definite classes or groups in which every plant is cross-sterile with every other plant of its class, but cross-fertile with every plant of every other group. STOUT (1916) has reported both sterile and fertile combinations in Cichorium, but he has been unable to classify his data into groups. In *Verbascum phoeniceum*, SIRKS (1917, 1926 and 1927) was unable to find any divisions of his material into groups in his earlier generations, but the later data lent themselves to such an analysis.

Theoretical considerations

The explanations which have been offered to account for self-sterility in plants may be classified into two sorts, the philosophical and the genetical, with CORRENS' 1912 paper as the turning point. Prior to this, DARWIN (1876) and others had made numerous observations upon the problem but interpreted it from the point of view of natural selection rather than of the gene. As we are today more concerned with the latter, a detailed report of the earlier work may be omitted.

CORRENS (1912) selected two plants of *Cardamine pratensis*, crossed them reciprocally, and raised sixty offspring. These were then backcrossed, using the parents as pollinators, and about 700 pollinations were made among the offspring themselves. The results of the backcrosses showed that the 60 individuals could be divided into four like-sized groups of which one was fertile with both parents while another was sterile with one and fertile with the other. A third group was just the reverse of the latter, while the remaining plants were sterile with both parents. To account for this result, CORRENS hypothesized that inhibitors ("Hemmungsstoffe") were present in his material. One parent possessed the factor *B* which would prevent it from giving fertile results when crossed with any other plant bearing a *B*. The other parent possessed a *G* factor and behaved towards all other *G* plants as did the *B* parent towards all other *B* plants. In addition, the parent with the *B* factor possessed a *b* gene which was inactive, and, similarly, the constitution of the other parent was *Gg*. These four factors behave as multiple alleles,[1] so that when *Bb* and *Gg* are crossed

[1] This term, proposed by JOHANNSEN, is preferable to "allelomorph," which it should replace.

the four offspring types are *BG*, *Bg*, *bG*, and *bg*. The *BG* plants are sterile with both parents because of the *B* and *G* inhibitors. Likewise, parent *Bb* gives sterile results with group *Bg*, and *Gg* is sterile with *bG*. All other backcrosses are fertile. Several objections have been raised to this hypothesis, but in spite of them, it seems adequate to account for almost all CORRENS' results. BEATUS (1929) has more recently studied Cardamine, and his results are in agreement with those of CORRENS. He found a varying degree of pseudo-fertility, and this played a considerable rôle in causing CORRENS' results to be less sharply defined than those of some other investigators.

Many studies upon genera other than Cardamine have shown that CORRENS' hypothesis is not generally applicable to them. Among these plants may be listed the various self-sterile species of Nicotiana, and *Veronica syriaca*. These plants are self- and cross-sterile in a way that can be interpreted by a theory basically different from that of CORRENS, and which is generally called "the theory of oppositional factors." The first statement of this hypothesis was by PRELL (1921), but his paper lay unnoticed for several years. EAST and MANGELSDORF (1925) and FILZER (1926) subsequently independently formulated an identical theory, and PRELL'S paper was brought to light by SIRKS in 1926. This theory of oppositional factors depends upon a series of multiple alleles, any two of which may be present in the tissues of the style of a self-sterile plant. Each factor in the cells of the style affords a stimulus only to a pollen tube which bears a different factor, so that the male gametes carried by pollen tubes which bear factors similar to those of the style do not reach an embryo sac and have no opportunity to unite with an egg. For the sake of simplicity, and because it is more in accord with the terminology used by the Drosophila workers, superscripts rather than subscripts will be used in this paper to designate these multiple alleles. If a plant which bears the genes s^1 and s^3 is used to pollinate a plant whose constitution is s^1s^2, the s^1 factor in the style affords no stimulus to a pollen tube carrying an s^1 male gamete, but s^3 tubes are stimulated and succeed in reaching and fertilizing eggs, so that the progeny consists of two classes, s^1s^3 and s^2s^3. If now an s^1s^3 plant is self-pollinated, there is no pollen grain possessing a factor different from the factors appearing in the style, and there is a failure of self-fertilization. When the cross $s^1s^2 \times s^3s^4$ is made, both types of male gametes reach the eggs and unite with them, and four classes are found in the offspring, s^1s^3, s^1s^4, s^2s^3 and s^2s^4. None of these offspring possesses two factors identical with those of either of the parents, so that all backcrosses in this latter case should be fertile. In the first instance, when

there is one factor in common, the s^2s^3 offspring will be fertile with both parents, while the s^1s^3 sibs will be fertile with their mother but sterile with the father. According to this theory, then, the results of backcrossing are different from those obtained by CORRENS.

In Nicotiana, EAST (1913, 1915a) found few sterile crosses in the earlier generations, but the later ones showed a great increase in the proportion of sterile crosses. The actual presence of intra-sterile, inter-fertile classes was reported by EAST and PARK in 1917, and also by EAST (1917, 1918). This discovery was very important, for it was the behavior of these classes when intercrossed that enabled EAST and MANGELSDORF to formulate the theory of oppositional factors. In a study of the progenies of reciprocal crosses, ANDERSON (1924) demonstrated that while two classes were present in the offspring of each cross, only one was common to the two progenies. Further tests showed that the class of the mother was never represented in the offspring of a cross, and this situation was explained by EAST and MANGELSDORF (1925) when they announced their theory. By availing themselves of pseudo-fertility, these investigators obtained classes which were homozygous for one of these factors. An s^1s^1 class would therefore give fertile results when crossed by any plant heterozygous for self-sterility factors, but the reciprocal cross would be fertile only when the female did not possess an s^1 gene.

After there was no doubt that oppositional factors determined self-sterility in Nicotiana, efforts were made to discover, if possible, how many such multiple alleles there were. Seven were reported by EAST in 1927, and by the following year fourteen had been recognized. EAST and YARNELL (1929) have identified sixteen multiple alleles, of which fifteen are genes for self-sterility, while the sixteenth, called S_f, is a factor for self-fertility. An interesting confirmation of the theory of oppositional factors has been offered by BRIEGER and MANGELSDORF (1927), for they succeeded in demonstrating a linkage relationship between a self-sterility factor and a color factor such that the selection of self-sterility gametes seriously disturbed the expected color-factor ratios.

The situation in *Veronica syriaca* seems very much the same as that in Nicotiana. LEHMANN (1919) has reported that this species is completely self-sterile, and that a cross between two plants yields four intra-sterile, inter-fertile groups. Later results showed that some crosses resulted in only two such groups. As in Nicotiana, in this latter case, the class of the mother was always absent from the progeny, so that reciprocal crosses did not produce the same two groups. Such results led FILZER (1926) and

LEHMANN (1927) to the same hypothesis that was advanced by PRELL and EAST and MANGELSDORF. LEHMANN (1927) checked this theory by an entirely different method. He showed that if the offspring of two plants I and II were crossed with those which originated from the cross III×IV, several possibilities might happen, depending upon the constitutions of the four P_1 plants. If they were *ab*, *cd*, *ef*, and *gh*, all the combinations of the inter-family crosses of the offspring should be fertile, and the same is true if the parents are *ab*, *cd*, *ef* and *cd*. If, however, the constitutions of the parental plants are *ab*, *cd*, *ed* and *ab*, one-eighth of the crosses should be sterile. He tested this by crossing together 51 plants and found but sixteen sterile crosses out of 800 combinations. A test showed that there were seven multiple alleles present in these 51 plants so that the great preponderance of fertile combinations was in accord with the hypothesis.

SIRKS (1926, 1927) showed that *Verbascum phoeniceum* follows the oppositional factor scheme with a slight modification. He assumes that s^1 pollen is incompatible not only in an s^1 style, but also in one which bears s^2 factors, while, inversely, s^2 pollen is compatible in an s^1 style. Likewise, s^3 pollen is incompatible in both s^3 and s^4 styles, while s^4 fails to function only in the latter. These assumptions are made to account for differences in reciprocal crosses. SIRKS (1927) has explained CORRENS' (1916) work upon *Linaria vulgaris* by a similar assumption, maintaining that s^1 pollen is incompatible in s^4 styles as well as in s^1.

LAWRENCE (1930) has recently attempted to explain the behavior of CORRENS' material upon the basis of the theory of oppositional factors and the polyploid nature of *Cardamine pratensis*. He regards this species as an allotetraploid, and therefore assumes it has four self-sterility factors; these are oppositional factors similar to those of PRELL, EAST and MANGELSDORF, and LEHMANN and FILZER, rather than to those which CORRENS supposed were acting in Cardamine. Two of these factors are called "*S*" factors. The other two are identical in nature and action with the *S* factors, but the letter "*Z*" is used for them. The *S* and *Z* factors are duplicate factors, and the only difference between them is that a chromosome bearing an *S* factor always pairs at meiosis with the one which bears the other *S*, and similarly the two chromosomes in which the *Z* factors are located go together; but an S^1 factor will exert its oppositional effect upon a Z^1 factor as well as an S^1, and Z^1 will prevent the acceleration of a pollen tube bearing either Z^1 or S^1 factors. All the other factors behave in a like manner towards one another, so that S^2 and Z^2 may be called similar, as may also S^3 and Z^3, etc. LAWRENCE further showed that if CORRENS' *B* and *G* parents had the constitution $S^1S^1Z^1Z^2$ and $S^3S^4Z^3Z^3$, all the results of

crossing the parents onto the offspring could be explained according to table 1.

TABLE 1

A graphic representation of LAWRENCE'S *(1930) explanation for* CORRENS'S *backcrosses of parents to offspring.*

	$S^1S^2Z^1Z^2$ ♀	$S^1S^2Z^1Z^3$ ♀	$S^1S^2Z^2Z^3$ ♀	$S^1S^2Z^2Z^4$ ♀
$S^1S^1Z^1Z^3$ ♂	+	−	+	−
$S^3S^4Z^2Z^3$ ♂	+	+	−	−

A similar explanation was offered by LAWRENCE for SIRKS'S data. LAWRENCE points out that the tetraploid nature of *Verbascum phoeniceum* must be considered, and that the plants cannot be treated as diploids. Assuming these two pairs of oppositional factors and autosyndesis, it is unnecessary to suppose that the S^1 and S^3 genes are incompatible in S^2 and S^4 styles respectively. CORRENS (1928a) considers that there are two distinct types of inheritance of self-sterility, the "Cruciferen-Typus," such as will explain Cardamine, and the "Tubifloren-Typus," which is explained by the theory of oppositional factors. In another paper, CORRENS (1928b) calls these two types the "Cruciferen-Typus" and the "Personaten-Typus." The discovery that Tolmiea could not be grouped with either Cardamine or Nicotiana established the need of a further classification. This was met by BRIEGER (1929, 1930). He first divided self-sterility ("self-parasterility") into two chief divisions, the one phenotypically, the other genotypically determined. The first type includes only Tolmiea, which he (BRIEGER 1929) calls the "Saxifragaceen-Typus," while the genotypically determined class is subdivided into the Crucifer and Personate types. In the Crucifer type, the constitution of both diploid parents is determinative of self-sterility, whereas in the Personate type, the diploid constitution of the female and the haploid of the male are the determining factors. In other words, in the Crucifers, self-sterility is determined sporophytically, while in the majority of plants it is determined sporophytically in the female sex and gametophytically in the male.

These classifications of CORRENS and BRIEGER need revision since the publication of KAKIZAKI'S most recent paper (1930). This investigator worked upon the Cruciferous *Brassica oleracea*, but explained his results on the basis of the oppositional factor hypothesis plus a subsidiary factor which is "sympathetic" in its action. KAKIZAKI found self-fertile plants in families from crosses between two self-sterile plants, and these self-fertiles when selfed yielded both types in their progenies. To explain this, and

also other features which differed from those found by CORRENS, by EAST and by LEHMANN, KAKIZAKI supposed the presence of a normal series of oppositional factors, called S_1, S_2 and S_3, and another series of alleles, T_1 and T_2. These latter accelerate the growth of pollen tubes bearing like factors. The oppositional factors are epistatic to the sympathetic, but the latter in homozygous condition are epistatic to the S factors, when the S factors are heterozygous. KAKIZAKI's analysis of Brassica indicates that the type of self-sterility determination found by CORRENS in Cardamine is not appropriately designated the Cruciferae type, but perhaps should be renamed the "Cardamine-Typus."

Before the enunciation of the theory of oppositional factors, EAST and PARK (1917) and EAST (1918) considered that self-sterility acted sporophytically in both sexes. This, of course, has been rejected, as their later scheme is based upon the gametophytic character of the pollen. FILZER (1926) elaborated this point, maintaining that if it were sporophytic, $\alpha\gamma \times \alpha\delta$ should give four classes, $\alpha\alpha$, $\alpha\delta$, $\gamma\alpha$ and $\gamma\delta$, instead of the two which were obtained, $\alpha\delta$ and $\gamma\delta$; however, it seems rather that such a cross should be sterile and thus yield no intra-sterile, inter-fertile classes, for the α gene in the style would prevent fertilization of the eggs by pollen of a plant which also possessed the α gene. Thus, by CORRENS' hypothesis, a *BG* or *Bg* style will prevent a union with pollen which bears the *B* factor. If the sporophytic character prevails, the allele of the *B* gene also would not act in such a style, even though it in itself were not opposed by any stylar gene. Therefore, the α factor in the male plant in FILZER's cross would prevent its allele from uniting, and the indicated cross would be sterile. If the oppositional scheme is operating, the *B* gene in the male would not affect its allele, and $Bg \times BG$ would be fertile, giving *BG* and *Gg* offspring.

A few of the 16 multiple alleles reported by EAST and YARNELL (1929) control pollen tubes which grow so slowly that no combination of stimulating subsidiary genetic factors plus the most favorable environment would cause them to grow sufficiently fast to produce seeds after a selfing. Others of these genes may be intermediate in their influence upon the growth rate of the pollen tubes, between these and the accelerating S_f factor. Some self-sterility alleles may give slight fertility under ordinary conditions, while others may give some fertility in an optimum environment only. It is suggested by these investigators that each of these 16 alleles may be characterized by a growth rate peculiar to itself, and that each may differ from the others by definite mutation quanta.

Extensive work has been carried out for a number of years by STOUT

in *Cichorium Intybus*, but his material is difficult to analyze, and no genetic explanation has been offered. STOUT (1916) obtained self-fertile plants from crosses between self-sterile ones, and found that self-sterility appeared in different degrees of intensity in different plants. On the basis of the oppositional factor hypothesis, STOUT (1927) suggests that his self-fertile plants might be due to a mutation of one of the genes to s_0.

Materials and methods

The genus Capsella (Bursa) has been under investigation at PRINCETON UNIVERSITY since 1915, and preceding that at the STATION FOR EXPERIMENTAL EVOLUTION since 1905; many thousands of plants have been raised in pedigree cultures during this period. An extensive report of much of this work has recently been published by SHULL (1929), who has recognized in his material ten species and two subspecies, and these fall into two groups with respect to chromosome number. Chromosome counts in eight species and one subspecies have been made by HILL (1927), and these corroborate the breeding investigations as to these two groups. If eight is taken as the basic chromosome number, one group is seen to be diploid and the other double-diploid or allotetraploid. Four of the species belong to the 16-chromosome group, while the other six with the two subspecies contain 32 chromosomes. It is in only one of the diploid species, *Capsella grandiflora* Bois., that self-sterility occurs. The other species in the diploid group are *C. rubella* Reut., *C. tuscaloosae* Shull and *C. Viguieri* Blaringhem, and these, as well as all the members of the 32-chromosome, or *bursa-pastoris*, group, are completely self-fertile or nearly so. Crosses between *C. grandiflora* and members of the double-diploid group are made usually with some difficulty, and if hybrids are secured, they are completely sterile; but the other three diploid species cross easily and naturally with the self-sterile species, and the F_1 plants are completely self-fertile. Naturally, it is with these four members of the *rubella* group alone that self-sterility and cross-sterility, and cross-fertility in relation to cross- and self-sterility, have been investigated.

The original *grandiflora* material for these cultures was received by Doctor SHULL in 1914 from Doctor G. BITTER, Director of the BOTANICAL GARDEN at Bremen, Germany. In 1925, additional seeds were received from the BROOKLYN BOTANIC GARDEN at Brooklyn, New York, which in turn had received its cultures the preceding year from the BOTANICAL GARDEN at Triest, Italy. As these two different lots proved identical in every respect except that the Triest material had the leaf-lobes generally more tenuous, no effort is made here to distinguish between them.

The procedure adopted is practically the same as that outlined by SHULL (1929). All pollinators were kept under waxed paper bags, as were the racemes upon which the crosses were made. Generally, from six to ten flowers were pollinated for each cross. Only those flowers were castrated which seemed certain to open the next day, and as these seldom numbered more than three, the making of a cross involved several days. In the self-fertile species, plants which were used for seed for the following year were always kept under bags, and no plants were raised at any time from unguarded seed. A number of pedigrees were studied which were F_2 and F_3 hybrids from a cross between *C. grandiflora* and one or another of the self-fertile species of the *rubella* group. These were found to split into self-fertiles and self-steriles, and it was soon seen to be unnecessary to bag the plants to determine to which class they belonged, because the number of insects in the greenhouse, at least during the winter months when these plants came into bloom, were so few that while a self-sterile segregate occasionally had a few enlarged capsules, there was no difficulty in distinguishing it from its self-fertile sibs. Figure 1 illustrates a typical *Capsella grandiflora* plant. It will be noticed that most of the capsules are small, but three are seen to be quite large. As the plant was not kept under a bag, these capsules arose either from an out-cross or from pseudo-fertility. Figures 2 and 3, however, show almost all the capsules well developed. The former is of the species *rubella* and the latter is a *tuscaloosae* plant. About ten days or two weeks after a cross has been made, it is very easy to determine whether it succeeded or not. If it is sterile, the ovaries form into tiny triangular capsules, scarcely more than two millimeters wide at the widest part; but if the combination was fertile, the capsules are enlarged many fold. In figure 4, the cross at the left, $29(3)\times29(1)$, was highly successful, for nine large, full capsules can be identified, whereas cross $30(6)\times30(1)$, at its right, was undoubtedly completely sterile. Seed counts were made for almost all crosses, and in each case they merely corroborate the observations on the capsules. When making the seed counts, the placentae are held edgewise on the stage of a binocular microscope, and the number of thickened funiculi on each side are counted. Each thickened funiculus represents a developed seed.

The author wishes at this point to express his sincerest thanks to Doctor GEORGE H. SHULL for the facilities and aid which were given him. Unstinted use of the greenhouse was granted for this investigation, even though Doctor SHULL had to cut down on his own wants to do so, and in spite of the fact that the author had over two thousand plants at a time for several months a year. Even more important, however, were the en-

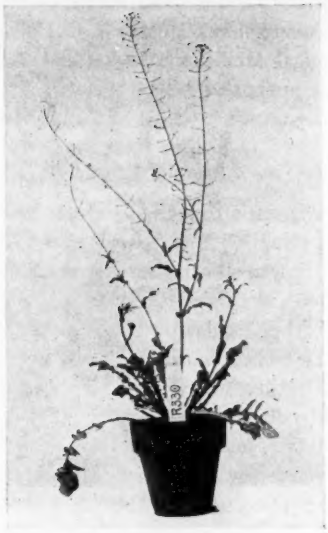


FIGURE 1.—*Capsella grandiflora.*

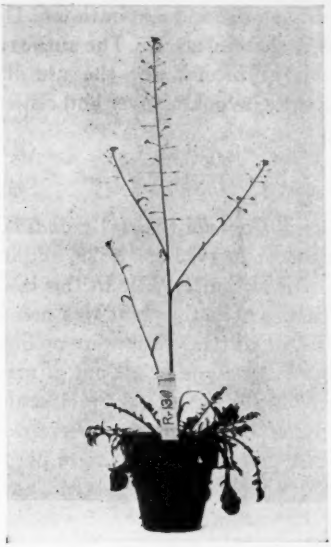


FIGURE 2.—*Capsella rubella.*

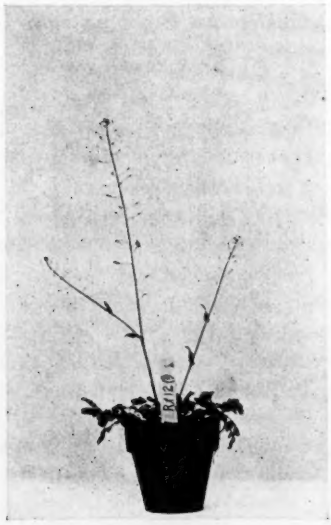


FIGURE 3.—*Capsella tuscaloosae.*

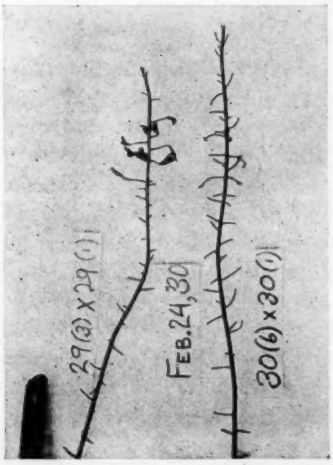


FIGURE 4.—A fertile (at left) and a sterile cross between plants of *Capsella grandiflora.*

couragement and inspiration of Doctor SHULL, and his constant readiness to give helpful advice. The author also wishes to thank Doctor E. G. CONKLIN of Princeton for the use of cytological facilities and for his great patience in looking over and criticizing the author's slides.

Observations

Self-sterility

That *Capsella* (*Bursa*) *grandiflora* Bois. is self-sterile has been reported by SHULL in 1923 and 1929, and in a joint paper with E. K. HALL and the present author (1932). In this last paper, it was shown that no difference existed whether a plant was merely bagged, its flowers self-pollinated by hand, or whether they were pollinated by other flowers on the same plant. Also, it was noted that out of many thousands of plants observed in this species, not one could be classed as self-fertile. There were some fertile capsules, to be sure, but these were so infrequent that there could be no doubt that they were due to pseudo-fertility. Nothing contrary has been observed in the 838 plants of *Capsella grandiflora* which have been studied in connection with this investigation. No condition has been observed similar to that found in *Cichorium Intybus* by STOUT (1916). There are no grades of self-fertility in *Capsella grandiflora*, for no plant has exhibited as many as five percent of fertile capsules. The results are so clean-cut that it can be stated beyond doubt that this species is completely self-sterile.

Cross-sterility

Intra-sterile, inter-fertile groups. In the preliminary paper (SHULL, RILEY and HALL 1932), it has been shown that each family can be divided into classes with reference to cross-sterility, and that this situation is fully in accord with that exhibited by most of the other self-sterile species which have been investigated by others. This report also showed that in general only two such classes were found in the offspring of any cross, although in a few cases there was an exceptional plant which was fertile with both groups into which its family split. The present investigation includes many more families. The chief conclusion is the same. Almost all families split into two groups only. In addition to the two main groups, four families also contained a group of one or more plants which were sterile with both the two main groups while one family contained a third group which was fertile with both the other two. Three families possessed all four such groups, and it is possible that more families might be included in these categories, but in some families the plants were tested by one pollinator

only. In general, however, only two classes are present in a family; whether these exceptional groups, which are usually much smaller in size, represent seed contamination, whether other phenomena cause their appearance, or whether they represent definite additional groups cannot be stated at the present time. If they are actually distinct classes, then some families split into four classes which bear a relation to each other such as CORRENS found in Cardamine, although in Capsella the doubly fertile and doubly sterile classes are much smaller in size than the other two.

In the first part of the investigation (1928–29) which forms the basis of the present report, six families were tested. Family 1 arose from a cross made by Doctor SHULL the preceding year between two plants of family 26674. Plant 1(1) was crossed onto eleven of its sibs, and 1(2) which was fertile with 1(1) was tested on five of these eleven. One plant was found to be sterile with plant 1(1) and fertile with plant 1(2), while three were just the reverse. Five other plants were sterile with 1(1) but untested with 1(2). One plant was sterile with both pollinators.

Family 2 (from the cross 26669(50)×(49)) showed five plants sterile with plant 1(1) and fertile with 1(2) or 1(11), which latter were sterile together. Two plants were just the opposite, that is, fertile with 1(1) and sterile with 1(2) or 1(11). Plant 2(8) gave sterile results with both pollinators, while four plants were fertile with both. The plants which are fertile with both pollinators, especially as they are quite numerous, must be considered a third class. Plant 1(1) has arbitrarily been called class A, plant 1(2) class B and these other plants class C.

Family 3 (from the cross 26664(35)×(31)) split into two A plants, and one which was fertile with both A and B, but untested with C. In family 4, which arose from the cross 26502(7)×(6), five plants were tested, but four by only one pollinator. Four were sterile and one was fertile with a B tester. The latter was fertile with A also, and was assumed to be in the C group. In family 5, six individuals were tested with all three classes. All were fertile with class A, while four were sterile with C and fertile with B. The other two were fertile with C and sterile with B. Family 6 (from 26321[2]×[1]) yielded five plants all fertile with the A group, of which two were sterile and three were fertile with the B pollinator. Unfortunately, class C was not crossed onto this family.

From the work of the year 1928–29, the presence of three intra-sterile, inter-fertile classes was definitely established, and it was shown that, with a few exceptions, each family splits nearly equally into two classes; this corroborates the preliminary report. Seed counts for these crosses are given in table 6 (for this and subsequent tables see Appendix). No generali-

zation as to the number of classes present in this species in the wild can be deduced from the fact that only three are present here, for all the pedigrees studied arose from only five original plants, and it is hardly likely that five families would contain more than three classes unless specially selected for that purpose, which these were not. Furthermore, even though more groups might have been present when the original lot of seeds was received, many may have been eliminated by close breeding, as no effort was made for a considerable time to identify classes and preserve them.

A different method of procedure was adopted for the pedigrees investigated in the second and third years of work. In these families, one plant was crossed onto a number of its sibs, (usually nine), and another plant of the same family which had given fertile results with the first was then crossed onto as many of the sibs as time would allow. Relations between the several families were then identified by crossing both classes of one family onto both in another. In the first half of these pedigrees, it was not possible to make as many of these inter-family crosses as was desirable, and even when they were made, they were made in only one direction. In the families studied in the year 1930–31, such inter-family crosses were made reciprocally. In table 7 (see Appendix), all intra-family crosses are listed with seed counts. A study of these will show the proportion of plants in each of the classes in each family. Almost all families split into two groups only. These are not of exactly equal size in most cases, but that is very probably due to the small size of the populations which were used. Two families, 10 and 30, were composed of but one group each. In family 39, eight plants were sterile with plant 39(1) and were fertile with 39(5); but plant 39(9) was fertile with both pollinators. This was no accident, for both crosses upon this plant were repeated and gave the same result. Some inter-family crosses were made among families 7 through 33 to determine group relationships. The families subsequent to these were studied in a different manner with reference to the parentage, and will be discussed under the appropriate sub-headings of the section on cross-sterility. In table 3 (page 249) these earlier inter-family crosses are tabulated.

Before studying inter-family relationships, it may be well to mention reciprocal crosses. It was early believed that reciprocal crosses were alike as to sterility or fertility in Nicotiana. This view was expressed by EAST (1917, 1918) and by EAST and PARK (1917), and was undoubtedly correct at that time. Later, however, by pollinating young buds, classes were obtained which gave sterile results with certain normally produced classes when used as the male parent, but which were fertile when the cross was made in the other direction (EAST and MANGELSDORF 1925, 1926). SIRKS

(1917, 1926, 1927) found a difference in reciprocal crosses and in his later papers explained it, not as EAST and MANGELSDORF had done, but upon the basis that the s^1 factor is incompatible in an s^2 style, whereas the s^2 factor can function in an s^1 style. STOUT (1916, 1927) found this reciprocal difference in many crosses, but refuses to adopt the explanation of EAST and MANGELSDORF.

Over ninety reciprocal crosses have been made in Capsella. One cross 10(1)×9(2) was fertile, while 9(8) which was of the same class as 9(2) seemed sterile with 10(1) when plant 9(8) was used as a female. This latter cross was made late in the life of the female plant and it is possible that some type of true sterility entered into the problem to confuse the situation. In all other cases, a cross which was fertile in one direction was fertile in the other, and all sterile crosses gave sterile results reciprocally.

According to the theory of oppositional factors, a cross (barring homozygosis for one sterility factor) may split into either four or two intra-sterile, inter-fertile classes each one of which is fertile with all others. Inter-family crosses in Capsella gave different results, for no family splits into four equal-sized groups, and when four groups are formed they do not show the same inter-family relationship that would be expected from the four groups of the oppositional factor type. When a family splits into two groups because of oppositional factors, the class of the mother is always absent. Thus, if s^1s^2 is crossed with s^1s^3, the offspring are s^2s^3, which is fertile with both parents, and s^1s^3, which is sterile with the father and fertile with the mother. As Capsella is an annual, such backcrosses cannot easily be made. In the year 1930–31, the offspring of a cross were back-crossed directly onto the sibs of their parents, which method has been as effective in studying class relationships as crossing them with the parents themselves would have been. In the earlier work, however, inter-family crosses were made among twelve families and an attempt was made to establish a class relationship between parents and offspring by inductive reasoning. A study of table 2 will assist in following this reasoning. The column at the left indicates the family in question; the second column gives the origin of the family and the third column the intra-sterile, inter-fertile classes of both parents. As before, plant 1(1) is called class A, 1(2) is class B and 2(2) is class C. In the fourth column are placed the two main classes into which the family in question has split, based upon inter-family crosses made in that generation, arbitrarily assigning class L to plant 9(1) and plants with which it is sterile, class M to 9(2) and class P to plant 14(9). The next six columns show the classes of each of these families

in terms of the classes of the parents on the basis of the assumptions indicated at the top of each column.

TABLE 2

Showing the method used in attempting to identify the inter-fertile, intra-sterile groups in the families of one generation with those of the preceding generation.

					IF: L=B M=A P=C	IF: L=A M=C P=B	IF: L=C M=B P=A	IF: L=B M=C P=A	IF: L=C M=A P=B	IF: L=A M=B P=C
7	2(2)×1(1)	C×A	L	P	B C	A B	C A	B A	C B	A C
8	2(10)×1(11)	C×B	M	P	A C	C B	B A	C A	A B	B C
9	5(3)×2(12)	B×A	L	M	B A	A C	C B	B C	C A	A B
10	2(2)×1(2)	C×B	P		C	B	A	A	B	C
11	2(12)×1(11)	A×B	L	M	B A	A C	C B	B C	C A	A B
12	2(10)×1(1)	C×A	L	P	B C	A B	C A	B A	C B	A C
13	5(3)×2(10)	B×C	M	P	A C	C B	B A	C A	A B	B C
14	2(4)×1(2)	C×B	M	P	A C	C B	B A	C A	A B	B C
15	5(6)×4(5)	C×B	P	M	C A	B C	A B	A C	B A	C B
31	4(3)×1(11)	C×B	P	M	C A	B C	A B	A C	B A	C B
32	5(1)×2(12)	C×A	P	L	C B	B A	A C	A B	B C	C A
33	5(1)×4(5)	C×B	P	M	C A	B C	A B	A C	B A	C B

There are six possible combinations by which A, B and C may coincide with L, M and P. The appropriate combination for each column is placed above it, and, within the column, A, B and C are substituted for letters in column 4 upon the basis of the equalities expressed in the heading of the column. This is done on the assumption that the three classes found in the families of the second generation are the same as the three classes of the parental generation in some arrangement. According to the oppositional factor hypothesis, a cross A×B would give classes B and C because the maternal class would be absent. A study of the table shows that this holds in some cases. In column 6, family 7(C×A) gives A and B; but in family 11, using the same substitutions of A, B and C for L, M and P, A×B gives A and C, and this disagrees with the oppositional factor scheme. In fact, no combination of substitutions will fit the oppositional factor hypothesis in all cases. However, a thorough study will reveal that if the two classes in the progeny of a cross are the *same* as the two classes to which the parents of the cross belonged, every case but one will agree when the substitutions are made in column 10. If L = A, M = B and P = C, and if any cross produces the same two classes only, there are eleven cases which agree and only one that does not. From these families, then, it can be concluded that the two main classes of a family are the same as those

of the parents, and that the class of the mother is not absent from the progeny. Studies described under the next section will furnish further evidence upon this point. In family 10 only one group was found, but only ten plants of this family were tested, and it seems not improbable that a larger population in the family might have brought to light a second group. So far as the group which is present is concerned, it behaves normally, so that there are no *fundamental* cases of disagreement in any of the twelve families studied.

Backcrosses. The life span of Capsella is short. This is disadvantageous for the genetical study of self- and cross-sterility, as doubtful results cannot always be rechecked by a repetition of the cross. A more serious difficulty is that the plants cannot be held over from one year to the next for purposes of comparing them with later generations. On this account, no actual backcrosses have been possible, but plants have been crossed onto the sibs of their parents, and this gives the same kind of information as would be secured from actual backcrosses. A portion of the seeds secured from the cross 2(10)×1(11) were sown in 1929, and ten plants were raised and labeled family 8. These plants split into seven of one group and three of another. In the following year, family 34 was raised from the cross 8(1)×8(5), and family 35 from 8(5)×8(1). In addition, the remaining seeds from the cross 2(10)×1(11) were sown, and these comprised family 56. Thus, the plants in family 56 were the sibs of the plants in family 8, and the two classes in family 56 must be the same as those in family 8. The relationship of the groups in family 56 to those in family 8 could not be determined directly as the plants of family 8 had died months before those of 56 came into bloom. Families 34 and 35 each gave two main intra-sterile, inter-fertile classes. The next step was to cross each of the classes of families 34 and 35 onto each class of family 56, reciprocally. If the oppositional factor scheme were operating, one class of family 34 should have been sterile with one of family 56 and fertile with the other, while the other class in family 34 should have been fertile with both classes of family 56. Such results were not obtained. Plant 34(3) was sterile with plant 56(1) and fertile with 56(3), but plant 34(1) was fertile with 56(1) and sterile with 56(3). Reciprocal crosses gave identical results. In family 35, plant (2) was sterile with plant 56(2) and fertile with 56(1), while plant 35(4) was exactly the reverse.

Similar to the above is the relationship of the progeny of the cross 5(3)×2(12) and the sibs of their parents. Eleven plants from this cross were raised in 1929 as family 9, and ten the following year as family 49. Simultaneously with these last were raised two families of offspring from crosses

of plants in family 9. Cross 9(2)×9(1) formed family 44, while family 45 came from the cross 9(3)×9(1). All these families yielded only two groups, and inter-family crosses exhibited the same relationship as in the previous case. One of the groups of family 44 was sterile with one of the groups of family 49 and fertile with the other, while the second class of family 44 was fertile and sterile, respectively, with the two classes of family 49. Family 45 gave identical results when crossed with family 49. Details of all these backcrosses may be found in table 9.

The origin of families 7 and 53 was the cross 2(2)×1(1). Family 51 came from 7(4)×7(1). Inter-family crosses between this and family 53 (the sibs of the parents) showed the presence of two similar groups. More extensive studies were made with family 52 (from 7[9]×7[1]) and family 53. Plants 53(1) and 53(6) both represented one class in family 53, while 53(3) was a plant of the other class. The first two plants gave sterile results with 52(1) and fertile with 52(2), while 53(3) was fertile with 52(1) and sterile with 52(2). Reciprocal crosses were the same. Table 9 lists also a number of other crosses between plants of these two families, and these merely give additional data. Similarly, in the previously described relations between families 44 and 49, 29 crosses were made using plants of family 49 as the mother and several plants of family 44 as the father. These all bear out the hypothesis that the offspring of a cross are generally of the same two groups as the parents, and that even when two additional classes are present, the two main groups are the same as the two main parental ones.

There can be no valid objection to using sibs of the parents instead of the parents themselves. Since any family normally splits into only two groups, it is not necessary to know, for example, whether it is plant 34(1) or plant 34(3) that is sterile with plant 2(10). The essential fact is that one of the two classes of the offspring is the same as one of the two parental classes, and that the other group in the progeny is identical with the other parental group. This is the only fact necessary to establish the contention that the class of the mother is not absent from the progeny, and that the same two main groups exist in both the offspring and the parents. It has been impossible to connect together the plants sown in one year with those of another. It might seem possible by making frequent sowings every month or so to establish a continuity of classes, but this is not practical, for Capsella does not develop well in our climate during the summer, as SHULL (1929) has pointed out.

Offspring of reciprocal crosses. In those species in which self-sterility is controlled by oppositional factors, the cross A×B gives groups B and C; the reciprocal B×A gives A and C; so that one and only one class is com-

mon to the progenies of reciprocal crosses. In this and subsequent sections, reference to the oppositional factor hypothesis will relate only to those crosses in which one and only one self-sterility allele is common to both parents, since this case, only, results in two groups in the progeny. Capsella has never been found to give four groups like those in Nicotiana which result from a cross of the type $s^1s^2 \times s^3s^4$. If it is true in *Capsella grandiflora* that the same two classes are present in the parents and offspring, two identical classes should also be found in the progenies of reciprocal crosses, for A×B would give A and B while B×A would also result in the classes A and B.

In the earlier work, the direct crossing together of the two families of a reciprocal cross was not made, but there are two instances of reciprocal crosses of the same two groups, although the individual plants were different. The results are given in table 3, and the letters used are those employed in table 2. It is seen that both A×B and B×A give groups A and B, and that B×C and C×B both give B and C.

TABLE 3

Offspring of reciprocal crosses.

FAMILY	ORIGIN	CLASSES OF PARENTS	CLASSES OF OFFSPRING
11	2(12)×1(11)	A B	A B
9	5(3)×2(12)	B A	A B
13	5(3)×2(10)	B C	B C
14	2(4)×1(2)	C B	B C

Later studies have been made by raising two families from the same parents crossed reciprocally, and then crossing each group in each family onto each class of the other. Table 10 gives seed counts for these inter-family crosses. Family 34 arose from the cross 8(1)×8(5), and the reciprocal cross formed family 35. Plants 34(1) and 35(1) were sterile, reciprocally, as were also 35(5) and 34(3). All other combinations were fertile. The reciprocal cross of 9(2) and 12(1), which formed families 36 and 37, gave identical results. When the offspring of 9(6) and 10(1) and its reciprocal were raised as families 38 and 39, however, a different situation was found. Plants 38(1) and 39(1) were sterile reciprocally, as were 38(6) and 39(9). In family 39, however, another plant was found which was fertile with both 39(9) and 39(1). This plant was likewise fertile with both classes of family 38. The author feels that plants 39(1) and 39(9) represent the two typical and characteristic groups of their family, for they give results consistent

with the two groups in most families. Plant 39(5), then, is exceptional. In family 38, also, an odd plant was found, for 38(2) was sterile with both main groups of its family. Unfortunately, this was not recognized until it was too late to use it in inter-family crosses. In the preliminary paper previously mentioned one case of reciprocal crosses was studied, and this also showed the presence of two identical groups in the progenies.

Families from a common mother and different fathers. Under this heading may be listed two types of cross. In each case, the female is the same plant, while the males are different plants; but in the one case, the males both belong to the same intra-sterile, inter-fertile class, while in the other, each male belongs to a different class. Two crosses illustrate the first situation: family 35 came from the cross 8(5)×8(1), while family 43 was from 8(5) ×14(9). A glance at table 8 will reveal that plant 8(8) (which was sterile with plant 8[1]) and plant 14(9) were sterile together. Therefore, it is assumed that all three were of the same group. Plants 35(2) and 35(4) were fertile together, as were 43(1) and 43(2). Plant 35(2) was sterile with 43(2), and the same is true of plants 35(4) and 43(1), as table 11 indicates. In all cases reciprocals gave the same results. This study shows that two common groups were present in the offspring of the two families. The same relationship occurs when family 36 (from 9[2]×12[1]) and family 44, which arose from the cross 9(2)×9(1), were intercrossed. Plants 9(1) and 12(1) where sterile together, reciprocally. This type of cross agrees with the theory of oppositional factors, but it is also in harmony with this general Capsella scheme, in which the two main classes of the offspring are the same as those of the parents.

In the second case of common-mother crosses, the fathers belonged to different groups as shown by the fact that they were fertile with each other. Six crosses of this kind were studied. As in the case of reciprocal crosses in the earlier work the relations were studied by relating the groups as in table 2 (page 246), but, in the later studies, the direct inter-family crosses were made reciprocally. Family 9 arose from the cross 5(3)×1(12), while family 13 came from 5(3)×2(10). The two fathers were of different classes. One group of family 13, represented by plant 13(3), was sterile with 9(3) but fertile with 9(6), while 13(8) of the other group was fertile with both 9(3) and 9(6). Thus it is seen that there is only one class common to the two families.

The relationship of families 7 and 8 was similarly studied. Family 7 came from a C×A mating, and gave classes A and C, while family 8, which arose from a cross C×B, gave class B, which was definitely established, and either class A or class C, but the identity of the second class

was not determined. Since class B is present in the second progeny and absent in the first, it is seen that the two groups were not the same.

In the next four crosses, identical results were secured. Plants 8(1) and 9(1) were of different groups. The cross 8(5)×8(1) gave family 35, while 9(1) crossed onto the same mother resulted in family 42. Plant 35(1) was reciprocally sterile with 42(1), but 35(6) and 42(3) were fertile together in both directions. Also, all other combinations of these four plants were reciprocally fertile.

Inter-family crosses between families 42 and 43, which had a common mother and fathers of different groups, also showed that only one class was common to the two families. Nothing conflicting with these results could be found from the inter-family crosses of families 39 and 59, or of families 32 and 33.

The six illustrations of this type of cross are consistent although one cross mentioned in the preliminary paper does not agree. These six are in accord with this general Capsella scheme, for if A and B are crossed together, classes A and B are present in the progeny, while a cross of plants of groups A and C would give groups A and C. There is only one class which is common to the offspring of these crosses. On the basis of the oppositional factor hypothesis, two common groups should be expected, according to the scheme:

$$s^1s^2 \times s^1s^3 = s^1s^3 \text{ and } s^2s^3$$

$$s^1s^2 \times s^2s^3 = s^1s^3 \text{ and } s^2s^3.$$

This evidence only strengthens the previous conclusions that the hypothesis evolved independently by PRELL, EAST and MANGELSDORF, and FILZER will not suffice to explain the genetics of self-sterility in *Capsella grandiflora*.

Crosses involving fathers of a common class, but different mothers. As in the last case, this study can be subdivided into two phases, depending upon whether or not the mothers belonged to the same intra-sterile, inter-fertile class. Four investigations are offered in which the mothers were of the same class. They are listed in table 12. These all show that two common groups are present and need not be gone into in detail. They agree with the oppositional factor hypothesis, and are also consistent with the general results so far obtained in Capsella.

Six cases in which the mothers belonged to different classes have been examined, but only three follow this general Capsella scheme and these three are also the only cases which are in accord with the oppositional factor

scheme. The first two cases are included in table 4. They both show the presence of one common group.

TABLE 4

Families with fathers of the same class and mothers of different classes.

FAMILY	ORIGIN	CLASSES OF PARENTS	CLASSES OF OFFSPRING
7	2(2)×1(1)	C A	A C
9	5(3)×2(12)	B A	A B
14	2(4)×1(2)	C B	B C
11	2(12)×1(11)	A B	A B

In full accord with this result are one case described in the preliminary paper and the more recently studied families 37 and 59. However, when family 39 was compared with families 42, 44 and 45, two common groups were found in each case. Seed counts of these crosses are given in table 12. To be in harmony with most of the results obtained in Capsella, the progenies of crosses which had fathers of a common class and mothers of different classes should have one and only one class in common. According to the oppositional factor hypothesis, the same result should be expected according to the scheme:

$$s^1s^2 \times s^1s^3 = s^1s^3 \text{ and } s^2s^3$$

$$s^2s^3 \times s^1s^3 = s^1s^3 \text{ and } s^1s^2.$$

Of the six Capsella crosses of this type which were made, three fit in with the general results from other crosses, while the other three do not. These latter three also do not conform to the oppositional factor scheme, but the first three are in agreement with it. It will be noticed, however, that in each case of disagreement plant 39(5) was one of the plants used in the inter-family crosses of the progenies. Three groups were found in family 39, of which plants 39(1), 39(5) and 39(9) were representatives. Some other crosses showed that plants 39(1) and 39(9) represented groups which behaved like the two groups generally found in a *Capsella grandiflora* family, and that the group of plant 39(5) was atypical. It is unfortunate that this irregular plant was used so extensively in inter-family crosses; when the true situation was realized, it was too late to repeat these crosses using plant 39(9) in place of plant 39(5). It seems clear that these three inconsistent crosses were inconsistent because an irregular plant was used in place of one of the typical groups.

Various inter-family relationships have now been discussed. Table 5 summarizes these, showing what classes should be expected in the offspring of crosses, given certain classes in the parents, according to the hypothesis of oppositional factors; it also lists the expected classes of such crosses according to the Capsella scheme set forth in this paper, that the two main groups of a family are the same as the groups of the two parents. This table also illustrates the number of cases in this Shepherd's Purse material which agree and of those which disagree, with the oppositional factor hypothesis and with the Capsella scheme, according to the various types of mating which were made in this study. There are 26 cases of agreement and three cases of disagreement with the general Capsella scheme, while nine cases are in accord with and twenty cases disagree with the oppositional factor scheme. If plant 39(5) is to be considered not a typical class, as seems highly probable, and if the crosses in which it is involved are disregarded, all the tested cases are strictly in harmony with the Capsella scheme.

RELATIONS BETWEEN SELF-FERTILE AND SELF-STERILE PLANTS

Review of the literature

The relationship between self-fertile and self-sterile plants has attracted interest in a number of species. In *Reseda odorata*, COMPTON (1912, 1913) found both kinds of plants, and concluded from their breeding behavior that self-fertility is a simple Mendelian dominant to self-sterility. Such a condition was seen to obtain in other plants also. BAUR (1911, 1919) crossed the self-sterile species *Antirrhinum hispanicum*, *A. molle*, *A. glutinosum* and *A. Ibanyezii* with the self-fertile *A. majus*, and concluded that self-fertility was dominant and that duplicate factors were involved. HERIBERT-NILSSON (1916) found that in rye self-sterility is dominant over self-fertility. STOUT's (1918, 1920, 1922) results led him to consider that neither was dominant; the proportion of self-fertiles was variable, and certain lines and families seemed to maintain certain different grades of self-sterility.

In Nicotiana, EAST (1917, 1923) and EAST and MANGELSDORF (1926) considered self-fertility to be dominant, but their views have more recently been modified. EAST and YARNELL (1929) found that the factor for self-fertility, present in *N. Langsdorffii*, is allelic to the oppositional factor series. When a plant bearing these factors for self-fertility was crossed with a plant homozygous for one of the self-sterility factors, the F_1 was self-fertile, and if this was then selfed, a completely self-fertile F_2 resulted. This self-fertility factor has been called S_f rather than F as previously, and the only way in which it differs from the self-sterility alleles is that

TABLE 5

A tabulation of the cases which agree and which disagree with the oppositional factor and the general Capsella schemes.

SCHEME	CLASSES OF PARENTS	CLASSES OF OFFSPRING	RELATIONSHIP	NUMBER OF CASES WHICH AGREE	NUMBER OF CASES WHICH DISAGREE
Backcrosses					
Oppositional factor	A and B	B and C	1 common group	0	6
Capsella	A and B	A and B	2 common groups	6	0
Reciprocal families					
Oppositional factor	A and B B and A	B and C A and C	1 common group	0	5
Capsella	A and B B and A	A and B A and B	2 common groups	5	0
Common mother, fathers of same group					
Oppositional factor	A and B A and B	B and C B and C	2 common groups	2	0
Capsella	A and B A and B	A and B A and B	2 common groups	2	0
Common mother, fathers of different groups					
Oppositional factor	A and B A and C	B and C B and C	2 common groups	0	6
Capsella	A and B A and C	A and B A and C	1 common group	6	0
Common father, mothers of same group					
Oppositional factor	A and B A and B	B and C B and C	2 common groups	4	0
Capsella	A and B A and B	A and B A and B	2 common groups	4	0
Common father, mothers of different groups					
Oppositional factor	A and C B and C	B and C A and C	1 common group	3	3
Capsella	A and C B and C	A and C B and C	1 common group	3	3

the rate of pollen-tube growth in a tube bearing this factor is faster. This factor produces tubes which grow so fast that no combination of inhibiting factors and poor environment will cause self-sterility. As has been shown previously, the sixteen S factors which have been discovered may each be characterized by a definite growth rate. This S_f factor is at the one extreme. If plants are mated which have the constitution S_fS_f with those which are homozygous for the self-sterility allele S_1, the F_1 is S_fS_1. When this is selfed, the S_1 pollen does not act because of the S_1 factor in the style but the S_f pollen-grain sends out a tube which grows so rapidly that seeds are produced. The F_1 is composed of two types of plants in equal numbers, S_fS_f and S_fS_1, and all are self-fertile. The discovery that no S_1S_1 class and only half the usual number of heterozygotes are present in the F_2 led EAST and YARNELL to conclude that no dominant-recessive scheme will express the relationship between self-fertility and self-sterility in Nicotiana, and that previous contrary results were caused by technical errors.

SIRKS's (1927) explanation for the apparent dominance of self-fertility in many plants considers that the absence of oppositional factors causes self-sterility, an explanation somewhat analogous to that of EAST and YARNELL. According to SIRKS, the F_1 of the cross $S_1S_2 \times S_0S_0$ is S_1S_0 and S_2S_0. Due to the S_0 factor, the F_1 is self-fertile; but the S_1 factor must also grow in the S_1S_0 style, apparently much faster than it would in an S_1S_2 or an S_1S_1 style, in spite of the presence of the S_1 gene in the S_1S_0 style; otherwise no 3:1 ratio would be possible in the F_2, which would be entirely self-fertile as in the case of Nicotiana.

Observations

Crosses between *C. grandiflora* and self-fertile species

As previously mentioned, the three self-fertile species which are in the same chromosome group as, and which hybridize readily with, the self-sterile plant investigated in this paper, are *C. rubella*, *C. tuscaloosae* and *C. Viguieri*. Up to this time, no difference has been observed in the behavior of any of these towards *C. grandiflora*, so it will be the rule in this paper to group them together and treat them alike.

SHULL (1923) has reported that crosses between *C. grandiflora* and *C. Viguieri* are completely fertile. In a later paper, SHULL (1929) also extended this to crosses in which the other two self-fertile species were used. In all cases, he found that the F_1 was completely self-fertile, while segregation occurred in the F_2 of such nature that self-sterility seemed to be a simple Mendelian recessive to self-fertility. LAWRENCE (1930), discussing incompatibility in self-sterile diploids, gives the impression that SHULL's

conclusions for Capsella are identical with the results obtained by EAST in Nicotiana. This needs a word of explanation. SHULL (1929) stated that self-sterility in Shepherd's Purse results from the interplay of three or more self-sterility factors which seem to behave in essentially the same manner as those in Nicotiana, Veronica and Verbascum. This paper was read in 1926 at the INTERNATIONAL CONGRESS OF PLANT SCIENCES at Ithaca, New York, and SHULL'S statement was based upon what was then known about these plants. Since that time, EAST and YARNELL (1929) have shown that the F_2 from a cross between a self-fertile and a self-sterile plant is entirely self-fertile, and does not split into a 3:1 ratio as EAST (1917, 1918) had previously reported. SHULL'S statement, therefore, was not intended to cover this point, as this feature of the oppositional factor hypothesis was not known at the time his paper was read. SHULL has never obtained such results, and (1929) has definitely shown that crosses between self-fertile and self-sterile Capsella plants yield a self-fertile F_1, and that when the latter is selfed, F_2 and F_3 generations result in which both self-sterile and self-fertile plants appear. SHULL, RILEY and HALL (1932) confirm these facts for the F_1 and report that the F_2 segregates into a ratio which more nearly resembles 1.5:1 or 2:1 than 3:1. No explanation was offered, and the authors state they prefer to wait until they have more data before considering the F_2 ratios to be other than 3:1. In the present investigation, the descendants of five crosses were studied, particularly to check this earlier result. The crosses were made by Doctor SHULL during the year 1927–28, and the seeds were given to the author in the autumn of the latter year.

The F_1 generation

When the *grandiflora* plant 26502(7) was crossed by a *rubella* plant of the family 26322, the cross was fertile and the average number of seeds per pollination was 14.1. The family raised from this cross was designated r3, and eleven out of twelve plants were found to be self-fertile when bagged. Family r4 arose from a cross between *C. grandiflora* (26502[6]) and a *rubella* plant of family 26322, and the one F_1 plant which was bagged was self-fertile. The average number of seeds from the thirteen pollinations of the original cross was 14.2. When *C. tuscaloosae* was crossed onto *grandiflora* (26502[6]×26323) the seed average for seventeen pollinations was 18.5, while the cross 26502(7)×26323 gave 14.3 for the average seed count of fourteen capsules. The progeny of the first cross was called family t1, and of this, three plants were bagged. All were self-fertile. Family t4 originated from the other cross, and ten out of eleven plants of that family

were self-fertile. These F_1 populations were very small because it was felt that the self-fertility of the F_1 from this type of cross had previously been sufficiently demonstrated; these plants were bagged primarily for seed for subsequent generations. The only peculiar feature of these F_1 families is that one self-sterile plant was found in each of two families. The cause of this is unknown, and the failure to set seed may possibly have been due to some form of true sterility. Family v1 arose from the cross 26502(7) by a plant of family 26324. The female parent was a pure *C. grandiflora* plant while the male belonged to a family which was an F_2 from a cross between *grandiflora* and a segregated *Viguieri* from a still earlier cross between *grandiflora* and *Viguieri*. The fact that the F_1 split into approximately a 1:1 ratio instead of being all self-fertile indicated the heterozygous nature of the pollen parent. Being of the nature *Ss*, and crossed to an *ss* plant, the F_1 split as expected into 1 *Ss* (self-fertile) to 1 *ss* (self-sterile). The pollen-parent was undoubtedly heterozygous for self-fertility as the many previous crosses between pure *Viguieri* and *grandiflora* yielded perfectly self-fertile F_1 generations.

The F_2 generation

More extensive work has been done with this generation than with the F_1 since it was hoped that it would decide whether the F_2 ratio should be interpreted as 2:1 or 3:1. Seventeen F_2 families have been raised from various selfings of plants of the five F_1 families. These seventeen are listed in table 13, which gives the origin of the family, the number of self-fertile and self-sterile plants into which it split, and the expected 3:1 and 2:1 ratios for each family. Four families were raised from four plants of the *rubella* F_1 family, r3. These split into 91:32, 31:9, 5:2 and 39:5, and undoubtedly indicate a 3:1 rather than a 2:1 ratio. This same is true of family r13, which arose from r4(1) selfed, for this gave 75:25. Selfings of three plants of family t1 yielded three F_2 families which split into 50:25, 57:17 and 32:18. The first is a perfect 2:1 ratio and the last is very close to it, but the second approximates a 3:1. Six plants of the F_1 family t4 gave F_2 families. Two of these are more nearly in accord with the 2:1, for they gave 34:16 and 33:17, respectively, but the other four closely resemble the 3:1 ratio. The proportion of self-fertiles to self-steriles in these four families was, respectively, 31:11, 36:14, 27:9 and 30:13. Four F_2 families involving the self-fertile species *C. Viguieri* were sown, but only three germinated. Two of these resemble a 3:1 ratio, but the third can be more easily interpreted as a 2:1 ratio, as can be seen from the table.

The F_3 generation

Viewing the F_2 generation as a whole, it is probable that the simple Mendelian ratio is the one which explains this material. The proportion of self-fertiles was much greater than that observed in the families reported in the preliminary paper, and the ratios conform rather to those found by SHULL (1923). F_3 generations were raised from two F_2 families. If one-third of the self-fertile F_2 plants of a family were homozygous for self-fertility factors, while the remainder were heterozygous, one-third of the F_3 families which resulted from that F_2 family should be completely self-fertile while the rest should segregate into self-fertiles and self-steriles. It was expected that if the F_2 ratios were 2:1 all the F_3 families should segregate. Unfortunately, both F_2 families of which the F_3 generation was studied were those which more nearly corresponded to a 3:1 ratio. It is hoped that, in the future, an F_3 generation can be raised from F_2 families which approximated a 2:1 ratio.

In the year 1930–31, ten families were raised from bagged self-fertile segregates of family v5, and seven were raised from the F_2 family, r7. In one of the latter only two plants were raised to maturity, so that only six families can be considered valid evidence among these *rubella* hybrids. When possible, 125 plants were raised in each family, as this was considered a sufficiently large number to establish whether a 3:1 ratio occurred. Of the six *rubella* hybrid families, one, r19, contained 110 self-fertile plants to no self-steriles. The other families segregated into ratios which can be interpreted as 3:1. The exact numbers are listed in table 14. When the ten families which resulted from selfings of the F_2 family, v5, were checked over, it was found that four were completely self-fertile, while the remaining six segregated. This evidence is a very conclusive demonstration that a simple Mendelian dominant-recessive explanation can be adopted for these hybrids. The F_1 is self-fertile, and the segregations in the F_2 and F_3 are typical of such a situation. In all cases of self-pollination in this material, the seed is obtained merely by bagging the plant or part of it and allowing the flowers to pollinate themselves naturally. Once the bags are placed over the plants, they are almost never removed until the seeds have matured and are ready to be collected.

Crosses among F_2 segregates

As the author has been concerned chiefly with establishing the main principles of intra-sterile, inter-fertile group relationships among self-sterile plants, and of hybrid ratios between self-fertile and self-sterile species, he has been unable up to this point to go into the many ramifica-

tions of the self-sterility problem, some of which undoubtedly would help to clear up the situation. For this reason, he cannot offer as many sib crosses in the F_2 families as would be desirable.

Forty-four crosses in six families were made using one self-sterile segregate in each family and pollinating some of its self-sterile sibs with it. The results of these crosses, with seed counts, are listed in table 15. In family v2, self-sterile plant (5) was crossed onto eight of its self-sterile sibs. In each case the result was sterility, and in only one of these crosses was more than one seed per capsule obtained. In family v3, plant (1) gave sterile results with six sibs. Plant r6(51) was sterile with eight self-sterile sibs, and r13(6) showed no fertile results when crossed onto nine other self-sterile plants of that family. With seven self-sterile sibs, t10(11) gave sterile results, while with one other, an average of 2.7 seeds was found from seven capsules, of which six were to some extent enlarged. Even in pure *grandiflora* crosses, however, an occasional sterile cross will yield an average as high as this, so that it is very probable that this cross should also be regarded as sterile, the seeds produced being attributed to pseudo-fertility. Plant t11(45) was sterile with seven of its self-sterile sibs. In a pure *grandiflora* family, seven or eight plants when crossed by a sib usually showed some crosses entirely fertile and the rest entirely sterile, or nearly so; but in the six hybrid families just studied only one cross showed any tendency to be at all fertile. It can be concluded, then, that in these F_2 hybrids only one intra-sterile, inter-fertile group is present in each family. In a few cases, a self-sterile segregate was crossed onto self-fertile sibs, but the number of these crosses is very small. In some cases the cross was sterile and in some fertile. These crosses are included in table 15.

Fourteen F_2 families were tested by intercrossing the self-sterile segregates. In some cases the cross was sterile and in others it was fertile, but, unfortunately, the crosses were made in only one direction. These crosses are listed in table 16.

A few crosses were made between pure *grandiflora* plants and these self-sterile F_2 segregates; they are not numerous, chiefly because the self-sterile species had practically run out by the time the hybrids, which had been sown later, had become sufficiently old to determine whether they were self-fertile or self-sterile. The crosses were not made reciprocally; they are listed in table 17.

When it is desired to determine whether a hybrid is self-fertile or self-sterile, it is usually necessary to wait until many flowers have pollinated themselves before it can be decided in which category the plant belongs. This is chiefly because in many cases a large number of the earlier flowers

are sterile even in normally self-fertile plants, a condition which is known as "proximal sterility." This type of sterility has no connection with self-sterility and is found in plants of most of the self-fertile species, although usually to a less extent than is often seen in the hybrids. In the F_2 self-fertile hybrids, many plants showed a large number of sterile capsules distributed throughout. The proportion is larger in the F_2 plants than it is in the self-sterile species, and is still greater in the F_3. However, in such doubtful cases, the practice is to bag the plant so that one can be sure that the fertile capsules have not been the result of insect visits. The proportion of sterile and fertile capsules in the plant is then determined. When only a few such capsules develop, the plant is regarded as self-sterile with a high degree of pseudo-fertility.

Besides the inter-specific crosses which were mentioned above, some other families were studied which were supposed to be reciprocals of these, but which proved not to be hybrids at all. The flowers of the self-fertile diploid species, especially *rubella* and *Viguieri*, are extremely small, and when these plants are used as female parents, special care must be taken to castrate them before the anthers begin to open to discharge the pollen. Doctor SHULL reports that it has often happened in his experience that hybrid families in which *C. rubella* has served as the maternal parent have shown an admixture of purebred *rubella* plants with the F_1 hybrids due to accidental self-pollination. It is also possible that the pollen tubes of *grandiflora* grow more slowly than do those of the self-fertile species. The nature of the morphological characters of the F_1 and F_2 generations from these crosses was such as to show clearly that they were the pure self-fertile species and not hybrids with *grandiflora*. It is probable that self-pollination had taken place before the buds were castrated, and that by the time the *grandiflora* pollen was applied to the stigmas the pollen tubes from the self-pollination had advanced so far that all the seeds which developed were from self-fertilization. Since these families were either pure *rubella* or pure *tuscaloosae*, they were of no concern in the self-sterility problem, and have been omitted from the discussion and the tables.

PSEUDO-FERTILITY

Review of the literature

Up to this point, a self-sterile plant has been considered to be one which does not produce seeds when self-pollinated, even though both male and female gametes are normal. This definition may be modified, however, to include cases where some seeds are produced, for in many self-sterile species some plants set a few seeds when selfed. They are so few that these plants

cannot be confused with self-fertile ones. In a self-fertile plant of Capsella usually 80 percent or more of the ovules develop seeds, while in the most fertile of self-sterile plants the number of developed seeds rarely exceeds five percent of the available ovules. These few seeds that are produced are due to a phenomenon known as "pseudo-fertility." This condition is found in cross-sterility as well as in self-sterility; it may occur under what seem to be normal environmental conditions, or it may be brought about at will in some plants by selfing them late in the flowering season or by pollinating young buds.

In 1917, EAST and PARK reported that, when the pollinations were effected late in the flowering season of the female plant, some seeds developed from crosses which were expected to give sterile results. This type of pseudo-fertility received the name "end-season fertility." Other investigators have found this phenomenon in their material, but it must not be understood that all self-sterile plants show end-season pseudo-fertility.

Another method of producing seeds from normally self- or cross-sterile combinations, is to pollinate young buds before they open. This has been reported by EAST (1923, 1928) for Nicotiana, and SIRKS (1926) showed that young buds of *Verbascum phoeniceum* could be selfed at about the time of opening. In Nicotiana, SMITH (1924) found that 68 percent of the self-pollinations of unopened flowers were successful. In the flowers which had opened most recently, the percentage dropped to only 16, while no pseudo-fertility was recorded for the next oldest flowers. The same author (SMITH 1926) showed that the pseudo-fertility of *Nicotiana alata-N. Forgetiana* hybrids was of the same order as self-sterility rather than true self-fertility. In a variety of Petunia, BROOKS, WALSH and FERGUSON (1930) found a strain which they consider self-sterile but which would produce seeds in 87 percent of the flowers when self-pollinated at the time of the opening of the flowers and up to ten hours thereafter. After the flowers had been open for from 24 to 48 hours this ability to self-fertilize was lost. When these tests were repeated upon clones of these plants three years later, about 89 percent of the pollinations were successful even when made later than 48 hours after the flowers had opened. KAKIZAKI (1930) demonstrated that in *Brassica oleracea* bud pollinations of self-sterile plants increased the fertility to a considerable degree, except when the plants were too young. No amount of premature pollination would cause pseudo-fertility in completely self-sterile plants of species of Lilium, Brassica and Cichorium, according to STOUT (1927), while in the self-fertile plants of these same varieties, a full amount of seed was set without recourse to bud pollination.

Of even more importance than the discovery of pseudo-fertility is a study of the hereditary behavior of plants raised from seeds obtained in this manner. EAST and his co-workers have been largely responsible for determining this, at least in plants which accord with the oppositional factor hypothesis. EAST (1919) raised a family from seeds obtained by pseudo-fertility and found that it was entirely cross-sterile *inter se*, and did not split up into classes. EAST and MANGELSDORF (1925, 1926) followed this up and showed that plants obtained in this way were homozygous for one oppositional allele. When an S^1S^2 plant was selfed in the young bud, some of the offspring had the genetic constitution S^2S^2 and some were S^1S^1. The latter were fertile as females with S^2S^3, S^1S^3 and S^1S^2 plants and were fertile as males with the first of these three classes, but they were sterile when crossed onto the other two groups. The discovery of these homozygous plants was fortunate for it enabled the investigators to maintain pure lines of self-sterile plants whose genetic constitutions were known and which could be used in analyzing other self-sterile Nicotianas. It was by means of these homozygotes that EAST and YARNELL (1929) were able to identify the oppositional alleles in new material.

A further advance towards solving the nature of pseudo-fertility was made by BRIEGER (1927a). In a family of *N. Sanderae* hybrids, a plant of the constitution S^1S^3 appeared which was completely self-fertile. As a male, it behaved as a normal self-sterile S^1S^3 plant, but as a female it was fertile to S^3 pollen. Selfed seeds of this plant gave rise to progeny of which some were normal while some behaved after the manner of this anomalous plant. BRIEGER explained this by assuming the presence of a factor *P*, which causes pseudo-fertility, is linked with the oppositional factors, and prevents the opposing action of the factor with which it is linked. In a later paper (BRIEGER 1927b) he changed his views somewhat.

Observations

In the preliminary Capsella paper, it was reported that occasionally a few capsules which have enlarged to some extent will be found on a *C. grandiflora* plant which has been bagged. The number is extremely small, as probably 99 percent of the self-pollinations of this species are entirely unsuccessful. The few capsules which are enlarged contain at most only a few seeds and are due undoubtedly to this phenomenon of pseudo-fertility. In crosses, too, many pollinations are not fertile but, nevertheless, are not completely sterile. A perusal of the tables which give seed counts for crosses and which occasionally list the number of fertile and sterile capsules in a self-pollination will show that often a cross will have an aver-

age of around one seed per capsule. Fertile crosses usually have ten or more so that there can be no hesitancy in considering the others as cross-sterile but pseudo-fertile. While these seed averages show the degree of pseudo-fertility, they may be misleading if the whole situation in regard to them is not understood. In checking over the seed envelopes in which all seeds of all crosses and self-pollinations are kept, and on the backs of which is written the number of seeds in each capsule, the author noticed that there was a tendency for the capsules which contained some seeds to be those which were castrated on the last day of that operation. When a cross is started, those buds are emasculated which appear to be certain to open on the succeeding day. One cannot be sure of these, and often one might be castrated which would not open until two days later. If this occurs again on the second and third days of castration, the largest two or three buds will be castrated the fourth day, but while larger than the rest, they might not be ready to open for two or three days. If occasionally such buds are castrated and pollinated, they will be in reality bud pollinations, and the pseudo-fertility which occurs in those capsules will be analogous to that by which EAST gets homozygous families. Not all the pseudo-fertility found in Capsella may be accounted for by these inadvertently made bud pollinations, but it is very probable that much of the pseudo-fertility found in these crosses is due to this cause. It seems likely that this would also account for the high proportion of pseudo-fertility found in crosses as compared with self-pollinations.

To test whether bud pollinations will produce pseudo-fertility in Shepherd's Purse, and also whether it will occur when old flowers are pollinated, as KAKIZAKI demonstrated for another Crucifer, one cross which had previously been demonstrated to be sterile was repeated. The cross 34(6) $\times$34(1) was sterile. Later, while the plants were still in vigorous condition, a raceme of 34(6) was selected, and all capsules were clipped off up to three below the oldest flower. This raceme had been enclosed in a bag preceding the operation. Following these three sterile capsules were five old flowers with typical sterile capsules already formed but with the petals still adhering. Four flowers not quite so old and with the petals rather open succeeded these older flowers. Three slightly younger flowers came next, and then three flowers that had opened that day. All these were castrated, and emasculations were made upon buds which followed these flowers. The first two buds would positively have opened the next day. The next three buds were smaller, and probably would not have opened for two days. Then three smaller buds and finally four even smaller than these were castrated. The remaining buds were so small that it is doubtful

whether they could successfully have been castrated, and these were pinched off. On the day after castration, all these capsules, flowers and buds were pollinated with a number of flowers of plant 34(1) which had been kept under a bag. Seed counts were later made of all the seeds in each capsule in each of these categories. To make this clearer, the author has listed the number of pollinations made upon the stigmas in the various stages, and following this is a record of the number of seeds in each capsule beginning with the lowest in each case:

3	Sterile capsules			0,	0,	0
5	Very old flowers	0,	0,	0,	0,	0
4	Slightly younger flowers		0,	0,	0,	0
3	Still younger flowers			0,	0,	0
3	Flowers opened on day of castration			0,	0,	0
2	Buds (open next day)				0,	0
3	Buds smaller than last			0,	0,	0
3	Buds still smaller			0,	0+,	1
4	Very small buds		1,	0+,	0,	0

0+ indicates that the capsule was enlarged to some extent but contained no seeds.

No plants have been raised from known bud pollinations so the manner in which they would behave is not known. It is possible that pollen might occasionally contaminate its own stigma while a castration is being made, and, if this happens to unite with one of its eggs because of pseudo-fertility, this might possibly account for a peculiar plant which is occasionally found in the pedigrees, although this is by no means a certain explanation for these odd plants, especially those which are fertile with both main groups of the family.

A number of attempts to pollinate flowers were made when the plants had seriously declined in vigor. In earlier work, especially, some crosses were made in only one direction. Later it was desired to test the plants with other plants or to make the reciprocal cross; but in these later cases when the second cross was made the plants had generally become rather old. The result of some of these cases was sterility when fertility had been expected. No end-season pseudo-fertility has ever been found in Capsella, the tendency being apparently in the opposite direction, namely, end-season *sterility*.

PHYSIOLOGY

Review of the literature

Up to this point, self-sterility has been considered chiefly in its genetical aspects, but it has a physiological basis through which the genetic factors act. It was early shown by JOST (1907) that when a stigma is pollinated with pollen from a plant with which it is sterile, the pollen germinates and the tubes grow down through the style, but that their growth rate is much slower than that of the tubes in a compatible mating. CORRENS (1912) corroborated this statement. EAST (1915a) showed that in sterile Nicotiana crosses the flowers drop off unfertilized from eight to eleven days after pollination, but that when the cross is fertile, the growth rate of the pollen tubes is so great that gametic union takes place not later than the fourth day. EAST found that in selfed pistils the pollen tubes grew about three millimeters a day, with a slight acceleration as the tube progresses, and that the tubes never traversed above half the distance from stigma to ovary by the time the flower was ready to drop off. EAST and PARK (1918) plotted the growth rate and found that it was a straight line for sterile combinations, while the curve of a fertile cross resembles the first half of an autocatalytic reaction. EAST and YARNELL (1929) reported that the end of the pollen-tube growth curve in an incompatible mating was not strictly linear but showed that there was some retardation in rate of growth.

Apparently pollen-tube growth rate is not the only factor which will influence physiological incompatibility. CRANE (1925) reached the conclusion that in plums and cherries there is probably a definite block to self-fertilization. The tubes apparently grow at a like rate in both fertile and sterile crosses, and the different result seems due to causes which occur a short time after the tubes have penetrated the ovules. CRANE compared the reaction here to that found by MORGAN (1923) in Ciona. KRAUS (1915) and NAMIKAWA (1923) reached similar conclusions for the apple, that self-sterility is due to embryo abortion. These conclusions are contrary to those reached by KNIGHT (1917) who found differential pollen-tube growth in the apple.

Explanations for this differential pollen-tube growth rate have been numerous. EAST (1915a) suggested that secretions in the style offer a stimulus to pollen tubes from a plant with which it is cross-fertile, and that this secretion was a simple sugar, probably of the hexose group. Chemical substances were supposed to be present in the pollen grains, of the nature of enzymes of slightly different character, all of which except the one pro-

duced by the plant itself or one of a similar genetic constitution, could call forth this sugar secretion which gives the direct stimulus. EAST (1915b) further states that it is reasonable that the secretion in the style stimulates tubes from other plants rather than inhibits tubes from the same plant, and (EAST and PARK 1918) that, in a compatible cross, the pollen-tube nucleus produces a catalyser which causes the pistil to secrete substances which accelerate growth.

External factors are known to influence the growth rate of pollen tubes. STOUT (1923) discovered that less than ten percent of his *Brassica pekinensis* plants were highly self-fertile under normal conditions, but that when a generation was grown in small pots, which reduced the vegetative vigor, the percentage increased to around 65. EAST (1926) found that the growth rate in fertile combinations was very little influenced by the nurture of the plant, but that normal sterile matings were greatly affected by temperature and the relative lengths of day and night.

In 1926, EAST maintained that the reaction between the stylar tissue and a pollen tube in a fertile mating is mutual, and that no direct accelerating agent is called forth by these compatible tubes, for if it were it would stimulate the incompatible ones as well. It seems as if some easily diffusible substances pass from the style into the pollen tube and that there they cause the formation of the food necessary for growth. BRIEGER (1929) also considers the reactions to take place in the tubes themselves. SMITH (1924) and EAST (1927) have shown that so long as the tube reached the micropyle there is nothing to prevent self-fertilization and that the obstacles to seed-setting occur before the tube can get so far. Bud pollinations are an example of this, for in this case the tubes grow fast enough to cause fertilization before the flower withers. The advantage in time is not sufficiently great to be the cause of fertility in bud-pollination in Nicotiana, and it must follow that the tubes grow faster in buds than in mature pistils. EAST (1929) concludes that "the inhibiting substance or reaction does not occur, or is not so effective, while the flower is in the bud stage." In the same paper, EAST has also shown that subsidiary factors will affect the rate of tube growth but that they act only upon the mother; he further contends that each self-sterility gene possesses a characteristic growth curve in sterile matings.

KAKIZAKI (1930) maintains that his oppositional factors produce inhibiting substances which inhibit the growth in the style, and that when a cross is fertile the growth rate, which of course is faster, is due either to the absence of these inhibiting substances or to the presence of acceler-

ating substances which are produced by the sympathetic factors and which actively prevent the inhibiting action of the *S* alleles.

Observations

The author has very little evidence upon the behavior of pollen tubes in sterile and fertile combinations in Capsella. This was not due to a failure to realize the fundamental significance of the problem but to the lack of a technique which would permit tracing the tubes through the tissues of the pistil. No method which has been used has given satisfactory results, and the chief difficulty seems to be in the size of the material. Workers in Datura and other genera whose pistils are of a greater length than those of Capsella are greatly to be envied; the total length of the *Capsella grandiflora* styles rarely exceeds two millimeters, while that of the styles of the other three species studied is slightly smaller. BUCHHOLZ (1931) says, "In a technic for the study of pollen-tube growth, it is desirable to have, mounted on a single microscope slide, the entire strand of tissue thru which the pollen tubes grow. If this can be done, the study may become quantitative. Methods of dissection may achieve this end, which is defeated more or less by serial sections cut in paraffin." In Shepherd's Purse the author first attempted to make whole mounts, but the pistils are so tiny he was unable to dissect away the vascular tissue which surrounds the conducting strand. This vascular tissue is relatively large, and any attempt to see the tubes *in situ* was nullified by it. Recourse was then had to paraffine sections, which BUCHHOLZ has described as unsatisfactory but which gave slightly better results in this material than did whole, undissected mounts.

Pollinations of both fertile and sterile crosses were made and fixed at various intervals thereafter. The fixing fluid was generally the six percent formalin in 50 percent alcohol solution which BUCHHOLZ and BLAKESLEE (1927) recommend for pollen-tube studies in Datura, although Bouin's fluid was employed in some cases. Some slides were stained with magenta, but Delafield's haematoxylin with erythrosin, eosin or safranin was preferred. In almost all the fertile crosses or selfings, as in plants of the self-fertile species, the empty pollen-grain cases are apparent on the stigmas, and tubes may be seen emanating from them. It has been impossible in a single instance, however, to trace these tubes down to what is certainly their terminus. Due to the fact that the author used paraffine sections 10 or 15 micra in thickness, and that the tubes may not be straight, one cannot be sure that what is the end of the tube in a section is the real end, nor can he follow a tube through two or more sections. No stain which has

been employed in this study has stained the tubes any differently from the surrounding tissue, and this is a great handicap. Ungerminated pollen grains, however, stain very deeply and retain their roundish, somewhat triangular, shape, while germinated grains take little or no stain and appear to be shrunken. With a 1.2 oil immersion lens, the difference between these can readily be detected, and there can be no doubt that what appear to be tubes in the fertile crosses are such. No growth curves could be plotted, as it would be necessary to determine the ends of the tubes to do so, and this has not been found possible.

A study of sterile crosses or of self-pollinations of self-sterile plants shows different results. While the number of slides which have been studied is not large, not one has been found in a sterile combination in which the pollen grains are a mere mass of exine, or in which the tubes can be traced from them. In some slides many full, deeply-staining grains may be found adhering to the stigma, quite in contrast to the situation in fertile crosses.[2] In some of the slides, an outgrowth from the grain appears, like a small bud, but this is no longer than the thickness of the grain itself, and is just as deeply stained as the grains. Whereas pollen tubes have been definitely identified in stigmas of fertile crosses fixed two hours after pollination, none could be found larger than these little buds on stigmas of sterile combinations fixed up to four days after the pollen had been applied to the stigmas.

It is possible that further studies of many more slides prepared with a better technique will show that the pollen-tube growth is the same in Capsella as in Nicotiana and many other plants. At present, however, it seems as if there is little or no germination of grains in sterile combinations, although this conclusion must be considered as merely tentative.

DISCUSSION

In spite of the fact that the genetics of self-sterility has been worked out so completely and thoroughly in a number of plants, the author has had difficulty in formulating a theory which will explain all the known facts of Capsella, for this plant does not seem to fit in exactly with any theory proposed for other plants. It is obvious that the phenotypic explanation used for Tolmiea is not adequate, for certain Capsella crosses are sterile, which alone is sufficient to set it apart from this Saxifrage. This leaves

[2] In a paper which appeared after this went to press, STOUT (1931) reported that in the reactions of self-sterility in flowers of *Brassica pekinensis* he often found a low percentage of germination of pollen on the stigma.

the oppositional factor and Crucifer types as the two possibilities to be considered.

In species which are explained by oppositional factors, two plants which are cross-fertile will result in families, when crossed normally, which will be composed of either two or four intra-sterile, inter-fertile groups, and a plant of any one group will be fertile with those of all other groups. In Capsella, early studies showed that each family was composed of only two groups, and in determining this fact, the populations which were used were fairly large. When this more recent work was undertaken, only ten plants of every family were used, for it was considered fairly certain that both groups would be represented in a population of that size, and it was deemed more important to test as many families and to make as many inter-family crosses as possible rather than many intra-family crosses. In almost every family, two and only two groups were found; but very occasionally an odd plant appeared which was either fertile or sterile with both groups in this family. Very little attention was paid to these exceptional plants, as they were not numerous; but when the author was going over his data for the third year, he noticed several families in which both types of these anomalous plants were present. The behavior of these two exceptional types in the crosses which he made seemed to indicate a slight similarity between the families in which they were present and the results which CORRENS obtained in Cardamine. This analogy led the author to think that these exceptional plants were possibly more than a mere accident and that perhaps greater importance should be attached to them than had been. It was not ascertainable with certainty that these exceptional plants were either fertile or sterile with both pollinators until seed counts had been made; by that time, when the true significance of these plants was realized, it was too late to use them in crosses to test their behavior. However, what pollinations were effected made it certain that if these exceptional plants are regarded as separate types of classes, when four classes appear in a family they do not behave as they should if they followed the typical oppositional factor explanation, for each class is not fertile with all others.

When two groups only are present in a family of Nicotiana or Veronica, the class of the mother is not represented, and if backcrosses are made, only 25 percent of them will be sterile. A number of backcrosses of Capsella showed that when two classes are present, one is sterile with the father only, and the other is sterile with the mother and fertile with the father, and the percentage of sterile backcrosses is 50. Such a result is so different from the conditions in these other genera, that there is no doubt that the

simple oppositional factor explanation cannot be adopted. Another difference between Capsella and Nicotiana is the 3:1 segregation in the F_2 from a cross between self-fertile and self-sterile species of Shepherd's Purse, for such an F_2 in Nicotiana is entirely self-fertile. SIRKS's variation of the original oppositional factor hypothesis, assuming that pollen grains bearing certain factors were incompatible in styles which bore factors other than their own, also does not seem the basis of the true solution.

CORRENS' Crucifer type was then considered as a possible explanation. According to this theory, each plant contains one active inhibiting factor which will cause it to be sterile with any plant having the same active factor. The sporophytic character of both the male and female is determinative. In considering an explanation for Capsella, the author noted a possible resemblance to this type. CORRENS crossed two plants and the progeny contained four groups, of which one was sterile with both parents and one was fertile with both; a third was sterile with the mother and fertile with the father, while in the fourth, the situations were exactly reversed. This meant, then, that one of the groups in the progeny would behave as if it were the same as the mother, while another would seem identical with the father. Several types of test have shown that the two groups into which a Capsella family usually split are the same as the groups of the parents. For this reason they have been called throughout this paper the main or chief groups. When these two main groups are crossed onto any exceptional plant present in the family, both are either sterile or fertile with it. In CORRENS' cross, $Bb \times Gg$ gave $BG+Bg+bG+bg$. The middle two groups behave like the two parental ones; when crossed to their sibs, both should be fertile reciprocally with *bg* and sterile in both directions with *BG*. In this group relationship, Shepherd's Purse resembles Cardamine, except for the important point that in the latter the four groups are all of equal size. It can be definitely said that a Capsella family is composed of two groups which are the same as the parents. Whether these exceptional plants actually represent additional groups, or whether they are interlopers, cannot be stated without further work. If they are valid groups, the resemblance to CORRENS' family is striking in everything except the proportions of the four classes in the various families. A tentative theory is advanced which fits many of the results but which fails to account for the small size of the groups which can be compared to CORRENS' *BG* and *bg* plants.

Let it be assumed that self-sterility in Capsella is controlled by the sporophytic nature of both the parent plants involved in the cross. The reaction between the pollen tubes and the tissues of the style seems to be

one between cytoplasms and not nuclei; the cytoplasm of the pollen grains developed under the influence of the parental genotype; if a plant having the self-sterility factors *B* and *b* formed gametes which would be either *B* or *b*, the cytoplasm of those gametes could reasonably show the influence of the other self-sterility allele as well. This is in accord with CORRENS' theory. Let it be assumed also that there are three factors for self-sterility, s^A, s^B and s^C, which control the situation if they are present in either plant. If the two plants possess one or more like factors, they are sterile together, irrespective of the nature of the other self-sterility factors which either or both may possess. Allelic to these are other factors s^a, s^b and s^c, which are not inactive, as CORRENS hypothesized, but which determine the fertility of a cross if *neither* plant possesses one of the active factors. Also, a double dose of one of these factors acts as a single dose of one of the factors represented by capital superscripts.

If s^Cs^c represents the constitution of plant 2(2) and s^As^a that of plant 1(1), such a cross will be fertile because no like active factor is present in both parents. This cross formed families 7 and 53. If plant 7(9) had the constitution s^as^C and plant 7(1) was s^As^c, they would be fertile together. This cross was made and formed family 52, of which plant (2) was s^Cs^c and plant 52(1) was s^As^a. The other two possible classes were not represented. This, as stated before, is one of the weak places in this tentative hypothesis. These two plants of family 52 were crossed with the two groups of family 53 (the sibs of their parents). Plant 53(1) was s^As^a and was sterile with plant 52(1) because of the s^A factor, while plant 53(3), whose self-sterility alleles were s^C and s^c, was sterile with plant 52(2), because both plants possessed the factor s^C; 53(3) and 52(1) were fertile reciprocally, as were 53(1) and 52(2). The author has tested this scheme by assigning certain factorial combinations to his original plants and following them through the subsequent generations. In all the inter-family crosses studied, it is found that six crosses disagree out of 268. Intra-family crosses add many hundred more cases of agreement.

A feature of Capsella which does not appear in Cardamine is the dominance of self-fertility over self-sterility. It can be assumed that, allelic to the self-sterility factors, is a factor for self-fertility, which can be represented by the symbol *S*. EAST objects to the use of "dominant," and calls this self-fertility factor "S_f," but in Nicotiana it does not act like a dominant factor, whereas in Capsella it does.

When the *grandiflora* plant 26502(6), whose constitution was s^Bs^c, was crossed by a *tuscaloosae* plant which had two *S* factors, the cross was fertile.

Family t1 originated from the cross, and its plants were of the constitution Ss^B or Ss^c. One plant of the former type was selfed, and yielded an F_2 generation composed of $1SS{:}2Ss^B{:}1s^Bs^B$. If the oppositional factor scheme were in force, the F_2 would have been $1SS{:}1Ss^B$. Seventeen F_2 families were raised from different P_1 crosses and all gave this segregation. Owing to the fact that only three self-sterility alleles entered into the original crosses, only three types could be found among the F_2 segregates, when intercrossed. Three families were s^Cs^C, four were s^Bs^B and seven were s^bs^b. Plants of one type were reciprocally fertile with those of another except s^Bs^B and s^bs^b, which were mutually sterile because a double dose of *b* acts the same as *B*. At first it was thought that there were only two groups of three and eleven families, respectively; if this tentative theory is true, however, the large group can be divided up into two groups of four and seven plants, and this division follows from the symbols which were assigned to the original *grandiflora* plants.

In a number of instances, self-sterile segregates were crossed onto their self-sterile sibs, and all such crosses agree with the tentative hypothesis. When crossed onto their self-fertile sibs the same is true, but very few such tests have been made.

LAWRENCE'S (1930) recent paper is of great interest, as it shows that CORRENS' interpretation for *Cardamine pratensis* is not the only possible one. LAWRENCE follows the oppositional factor scheme, and shows that it will explain CORRENS' results if four oppositional factors are present, which is a plausible assumption, since Cardamine is presumably an allotetraploid. There are some species of Capsella which are double diploids, or allotetraploids, but all the species which have been studied in connection with the self-sterility problem are diploids, and an explanation based upon their diploid nature must be assumed as a basis for them. There have never been any signs of duplicate factors in the *rubella* group of Capsella, and there are no grounds, genetical or cytological, for supposing that the species belonging to this group are tetraploids. If duplicate factors for self-sterility are present in the diploid *Capsella grandiflora*, they would not give the result which LAWRENCE'S interpretation of CORRENS' results demands unless certain chromosomes always paired at meiosis, as do those in the tetraploid *Cardamine pratensis*, and it does not seem that this should be the case.

There is one serious objection to this tentative hypothesis and that is that it demands that four like-sized groups should be present in a family; in Capsella, most families have but two, and those families that may possibly be interpreted to contain four differ from the Cardamine families in

that two of the groups are so much smaller than the other two.[3] It is unfortunate that this situation cannot be accounted for. However, every cross in this material with the exception of about one percent can be explained by this tentative hypothesis, and this includes the relations between self-fertile and self-sterile species and the progenies which descend from such matings. Until further tests are made, however, the author prefers to consider this only a working hypothesis to be used as a guide in laying out experimental programs which will discover and test crucial matings.

The infrequency of appearance of plants which are either fertile or sterile with both main groups in their families suggests that these exceptional plants might have originated by crossing over. The author has not worked out a suitable explanation based upon this, but it is a possibility that must be given due consideration.

SUMMARY

1. *Capsella* (*Bursa*) *grandiflora* Bois. is a completely self-sterile species. No self-fertile individual has appeared in any family in the eighteen generations of this plant which have been studied in the greenhouse at PRINCETON UNIVERSITY. As in most self-sterile species which have been investigated, these plants may be divided into definite intra-sterile, inter-fertile classes, such that generally any plant is sterile reciprocally with any other plant in its group or class but is fertile in both directions with any plant belonging to another group. Such groups or classes have been found in the material studied in this investigation.

2. When two plants of different groups are crossed, a normal number of seeds is produced. The progeny of such a cross falls into two main classes which are identical with the classes of the parents. This fact was established by comparing the classes of a number of families with those of the families which included the parents.

3. Occasionally plants which give exceptional results are found in a family, in addition to the plants which belong to either of the two parental groups. In some cases, these plants are fertile with both of the two main

[3] After this paper had gone to press, BEATUS (1931) published a further report on *Cardamine pratensis*. The offspring of the cross $S6 \times S8$ split into four groups; two behaved like the parents, while the third was fertile and the fourth sterile with both parents. Contrary to CORRENS' results, but in accord with those of the present author, the two groups which behaved like the parents were larger than the two remaining groups. BEATUS' results differ from those in Capsella by the large amount of pseudo-fertility. In some families BEATUS found that many reciprocal crosses gave different results; this led him to the view that the gametophytic character of the male and the sporophytic character of the female are determinative of the fertility of the cross, while the explanation offered by CORRENS and the present author's tentative hypothesis are based upon the sporophytic character of both sexes.

groups of their family, and in some cases they are sterile with both. These exceptional plants were not found in every family and form a small proportion of the plants of the families in which they exist.

4. Evidence to prove that the two main groups of a family are the same as the groups of the two parents is of several sorts:

When the two main groups are backcrossed to the sibs of the parents of the family in which they are present, fifty percent of the crosses are sterile, while the remainder are fertile. One of the groups in the offspring family is fertile with one of the groups in the family composed of the sibs of the parents and is sterile with the other group in that family; the other group of the offspring family is sterile with the first mentioned group of the family of parental sibs, and is fertile with the second. Because the plant is an annual, it is impossible to make direct backcrosses, but evidence obtained by using the sibs of the parents instead of the parents themselves is deemed equally valid.

The progenies of reciprocal crosses, when intercrossed, show the presence of the same two groups in each family. Thus, fifty percent of such inter-family crosses are sterile.

When two crosses are made with one mother plant, but using different father plants of the same intra-sterile, inter-fertile class, two groups are found common to the two progenies.

When the same mother plant is given pollen from two plants belonging to different groups, only one intra-sterile, inter-fertile group is common to the two progenies, and only twenty-five percent of such inter-family crosses are sterile.

When pollen of a single father plant or of two plants of the same group is applied to the stigmas of two different mother plants which are of the same group, the progenies have two groups in common.

When the mothers of two such crosses are of different groups, only one of the two classes in one of the two progenies is identical with one of the two classes in the other progeny.

5. *Capsella rubella*, *C. tuscaloosae* and *C. Viguieri* are self-fertile species having the same chromosome number as *C. grandiflora* (HILL 1927). Crosses between any of these self-fertile species and the self-sterile *C. grandiflora* are fertile. The F_1 from such a cross is self-fertile, and the F_2 splits into three self-fertiles to one self-sterile. One-third of the self-fertiles breed true to self-fertility, while the other two-thirds split into a 3:1 ratio. All the self-sterile segregates of any F_2 family are cross-sterile among themselves. They seem to be sterile with some of their self-fertile sibs and

fertile with others, but too few tests have been made to establish the ratios, and this relationship needs further testing.

6. No end-season pseudo-fertility is exhibited by *C. grandiflora*, but, on the contrary, there seems to be an end-season true sterility in crosses which are fertile when the plants are in mid-bloom. There is a very slight amount of natural pseudo-fertility, and some fertile capsules may be obtained by bud-pollinations, although the number of seeds in these enlarged capsules is small.

7. Paraffine sections of pistils of self-fertile plants or of plants of a fertile cross show that the pollen has germinated and that pollen-tubes grow down through the styles; but these tubes have not been traced sufficiently far to enable one to draw growth curves. In several slides of sterile combinations, ungerminated grains were found on the stigmas, and no slides from such material showed a tube longer than the width of the pollen grain. More studies and a more satisfactory technique are needed if definite conclusions are to be drawn upon this point.

8. It has been shown that the theory of oppositional factors will not explain the inheritance of self-sterility in *Capsella grandiflora*, nor does CORRENS' Cardamine scheme fit without fundamental modifications which only further studies can fully elucidate.

LITERATURE CITED

ANDERSON, EDGAR, 1924 Studies on self-sterility. VI. The genetic basis of cross-sterility in Nicotiana. Genetics 9: 13–40.

BAUR, ERWIN, 1911 Einführung in die experimentelle Vererbungslehre. Ed. 2 viii+401 pp. Berlin: Borntraeger.

1919 Über Selbststerilität und über Kreuzungsversuche einer selbstfertilen und einer selbststerilen Art in der Gattung Antirrhinum. Z. indukt. Abstamm.- u. VererbLehre. 21: 48–52.

BEATUS, R., 1929 Über die Selbststerilität von *Cardamine pratensis*. Ber. deuts. bot. Ges. 47: 189–199.

1931 Zur Kenntnis der Selbststerilität von *Cardamine pratensis*. Z. indukt. Abstamm.-u. VererbLehre. 59: 285–348.

BRIEGER, F., 1927a Über genetische Pseudofertilität bei der selbststerilen *Nicotiana Sanderae* Hort. Biol. Zbl. 47: 122–128.

1927b Über die Genetik und Physiologie der Selbststerilität. Naturwissenschaften 15: 734–740.

1929 Die Selbststerilität der Blütenpflanzen und ihre züchterische Bedeutung. Züchter 1: 101–111.

1930 Selbststerilität und Kreuzungssterilität in Pflanzenreich und Tierreich. xi+395. Berlin: Julius Springer.

BRIEGER, F., and MANGELSDORF, A. J., 1927 Linkage between morphological characters and factors for self-sterility. Mem. Hort. Soc. New York 3: 369–371.

BROOKS, B. W., WALSH, L. B., and FERGUSON, MARGARET C., 1930 A cytological and a genetical study of Petunia. Amer. Nat. 64: 91–93.

BUCHHOLZ, J. T., 1931 The dissection, staining and mounting of styles in the study of pollen-tube distribution. Stain Technology **6**: 13–24.

BUCHHOLZ, J. T., and BLAKESLEE, A. F., 1927 Pollen-tube behavior with reference to sterility in Datura. Mem. Hort. Soc. New York **3**: 245–260.

CASTLE, W. E., 1896 The early embryology of *Ciona intestinalis* Flemming (L). Bull. Mus. Comp. Zool., Harvard University **27**: 201–280.

COMPTON, R. H., 1912 Preliminary note on the inheritance of sterility in *Reseda odorata*. Proc. Cambridge Phil. Soc. **17**: 7.

1913 Phenomena and problems of self-sterility. New Phytol. **12**: 197–206.

CORRENS, C., 1912 Selbststerilität und Individualstoffe. Festschr. med.-naturwiss. Gesell. zur 84. Versamml. deutsch. Naturforsch. u. Ärzte, Münster (Westf.) 186–217. Reprinted 1913 in Biol. Zbl. **33**: 389–423.

1916 Individuen und Individualstoffe. Naturwissenschaften **4**: 183–187, 193–198, 210–213.

1928a Ref. Über: Filzer, Paul, Die Selbststerilität von *Veronica syriaca*. Z. Bot. **20**: 296.

1928b Neue Untersuchungen an selbststerilen Pflanzen. I. *Tolmiea Menziesii*. Biol. Zbl. **48**: 759–768.

CRANE, M. B., 1925 Self-sterility and cross-incompatibility in plums and cherries. J. Genet. **15**: 301–322.

DARWIN, CHARLES, 1877 Effects of cross- and self-fertilization in the vegetable kingdom. viii+ 482 pp. New York: D. Appleton.

EAST, E. M., 1913 Inheritance of flower size in crosses between species of Nicotiana. Bot. Gaz. **55**: 177–188.

1915a The phenomenon of self-sterility. Amer. Nat. **49**: 77–87.

1915b An interpretation of self-sterility. Proc. Nat. Acad. Sci. Washington **1**: 95–100.

1917 The behavior of self-sterile plants. Science **46**: 221–222.

1918 Intercrosses between self-sterile plants. Mem. Brooklyn Bot. Gdn. **1**: 141–153.

1919 Studies on self-sterility. V. A family of self-sterile plants wholly cross-sterile *inter se*. Genetics **4**: 356–363.

1923 Genetical aspects of self- and cross-sterility. Amer. J. Bot. **10**: 468–473.

1926 The physiology of self-sterility in plants. J. Gen. Physiol. **8**: 403–416.

1927 Peculiar genetic results due to active gametophyte factors. Hereditas **9**: 49–58.

1928 The genetics of the genus Nicotiana. Bibl. genet. **4**: 243–320.

1929 Self-sterility. Bibl. genet. **5**: 331–370.

EAST, E. M., and MANGELSDORF, A. J., 1925 A new interpretation of the hereditary behavior of self-sterile plants. Proc. Nat. Acad. Sci. Washington **11**: 166–171.

1926 Studies on self-sterility. VII. Heredity and selective pollen-tube growth. Genetics **11**: 466–481.

EAST, E. M., and PARK, J. B., 1917 Studies on self-sterility. I. The behavior of self-sterile plants. Genetics **2**: 505–609.

1918 Studies on self-sterility. II. Pollen-tube growth. Genetics **3**: 353–366.

EAST, E. M., and YARNELL, S. H., 1929 Studies on self-sterility. VIII. Self-sterility allelomorphs. Genetics **14**: 455–487.

FILZER, PAUL, 1926 Die Selbststerilität von *Veronica syriaca*. Z. indukt. Abstamm.-u. Vererb-Lehre. **41**: 137–197.

FLORIN, R., 1927 Pollen production and incompatibilities in apples and pears. Mem. Hort. Soc. New York **3**: 87–118.

HERIBERT-NILSSON, N., 1916 Populationsanalysen und Erblichkeitsversuche über die Selbststerilität, Selbstfertilität, und Sterilität bei den Roggen. Z. Pflanzenz. **4**: 1–44.

HILL, SAMUEL E., 1927 Chromosome numbers in the genus Bursa. Biol. Bull. **53**: 413–415.

JOST, L., 1907 Über die Selbststerilität einiger Blüten. Bot. Ztg. **65**: 77–117.

KAKIZAKI, Y., 1930 Studies on the genetics and physiology of self- and cross-incompatibility in the common cabbage (*Brassica oleracea* L. var. *capitata* L.). Jap. J. Bot. **5**: 133–208.

KNIGHT, L. I., 1918 Physiological aspects of self-sterility of the apple. Proc. Amer. Soc. Hort. Sci. **1917**: 101–105.

KOELREUTER, J. G., 1761–1766 Vorläufige Nachricht von einigen des Geschlecht der Pflanzen betreffenden Versuchen und Beobachtungen, nebst Fortsetzungen. 1, 2 u. 3, 266 pp. Ostwald's Klassiker, Nr. 41. Leipzig: Engelmann.

KRAUS, E. J., 1915 The self-sterility problem. J. Hered. **6**: 549–557.

LAWRENCE, W. J. C., 1930 Incompatibility in polyploids. Genetica **12**: 269–296.

LEHMANN, E., 1919 Über die Selbststerilität von *Veronica syriaca*. Z. indukt. Abstamm.-u.Vererb-Lehre. **21**: 1–47.

1927 The heredity of self-sterility in *Veronica syriaca*. Mem. Hort. Soc. New York **3**: 313–320.

MORGAN, T. H., 1923 Removal of the block to self-fertilization in the ascidian, Ciona. Proc. Nat. Acad. Sci. Washington **9**: 170–171.

NAMIKAWA, I., 1923 Growth of pollen tubes in self-pollinated apple flowers. Bot. Gaz. **76**: 302–310.

PRELL, H., 1921 Das Problem der Unbefruchtbarkeit. Naturw. Wschr. **20**: 440–446.

SHULL, G. H., 1923 The species concept from the point of view of a geneticist. Amer. J. Bot. **10**: 221–228.

1929 Species hybridizations among old and new species of Shepherd's Purse. Proc. Int. Cong. Plant Sci. **1**: 837–888.

SHULL, G. H., RILEY, H. P., and HALL, E. K., 1932 A preliminary report on self-sterility in Shepherd's Purse. Unpublished.

SIRKS, M. J., 1917 Stérilité, auto-incompatibilité, et différenciation sexuelle physiologique. Arch. Néerland. Sci. Exactes et Naturelles B. **3**: 205–234.

1926 Further data on the self- and cross-incompatibility of *Verbascum phoeniceum*. Genetica **8**: 344–367.

1927 The genotypical problems of self- and cross-incompatibility. Mem. Hort. Soc. New York **3**: 325–343.

SMITH, F. F., 1924 Self-fertilization in Nicotiana. Science **59**: 461.

1926 Pseudo-fertility in Nicotiana. Ann. Missouri Bot. Gdn. **13**: 141–172.

STOUT, A. B., 1916 Self- and cross-pollinations in *Cichorium Intybus* with reference to sterility. Mem. New York Bot. Gdn. **6**: 333–454.

1918 Fertility in *Cichorium Intybus*: self-compatibility and self-incompatibility among the offspring of self-fertile lines of descent. J. Genet. **7**: 71–103.

1920 Further experimental studies on self-incompatibility in hermaphrodite plants. J. Genet. **9**: 85–129.

1922 Cyclic manifestations of sterility in *Brassica pekinensis* and *B. chinensis*. Bot. Gaz. **73**: 110–132.

1923 The physiology of incompatibilities. Amer. J. Bot. **10**: 459–461.

1927 Studies on the inheritance of self- and cross-incompatibility. Mem. Hort. Soc. New York **3**: 345–352.

1931 Pollen-tube behavior in *Brassica pekinensis* with reference to self-incompatibility in fertilization. Amer. J. Bot. **18**: 686–695.

APPENDIX

In the following tables the first column lists the female plants used in crosses. The second column contains male plants which were crossed onto these females. The first column after each cross indicates the number of

flowers pollinated, and this is followed by the number of capsules which were enlarged in whole or in part, then the average number of seeds per pollination, and finally a symbol to denote whether the cross was considered fertile or sterile. In some instances this cross and seed count are followed by a second and sometimes also by a third cross with their seed counts. In these cases the female is not repeated, and the columns which follow the male have the same connotations as the corresponding columns in the first cross. (inj) indicates that the raceme was injured and that it was impossible to obtain a seed count.

TABLE 6

Seed counts of crosses made in the year 1928–29. Families 1 through 6.

Family 1. Origin: 26674(18)×(13)										
1(1)	×1(1)	40	0	0	S	..	..	..	..	..
1(2)	×1(1)	5	5	6.6	F	×1(2)	52	0	0	S
1(3)	×1(1)	10	0	0	S	..	..	..	..	..
1(4)	×1(1)	11	5	1.5	S	..	..	..	..	..
1(5)	×1(1)	10	3	0.4	S	..	..	..	..	..
1(6)	×1(1)	11	3	1	S	..	..	..	..	..
1(7)	×1(1)	13	2	0.5	S	..	..	..	..	..
1(8)	×1(1)	10	0	0	S	×1(2)	8	7	3.4	F
1(9)	×1(1)	8	2	0.9	S	×1(2)	11	0	0	S
1(10)	×1(1)	8	7	7.3	F	×1(2)	7	1	0.1	S
1(11)	×1(1)	4	4	3.5	F	×1(2)	7	0	0	S
1(12)	×1(1)	7	4	2.7	F	×1(2)	8	0	0	S
Family 2. Origin: 26659(50)×(49)										
2(1)	×1(1)	6	0	0	S	×1(2)	6	5	13	F
2(2)	×1(1)	9	9	13.6	F	×1(2)	10	10	13.5	F
2(3)	×1(1)	9	0	0	S	×1(2)	8	7	9.3	F
2(4)	×1(1)	9	8	7.1	F	×1(2)	10	10	9.5	F
2(5)	×1(1)	8	7	5.6	F	×1(2)	8	4	4.9	F
2(6)	×1(1)	8	8	8	F	×1(11)	7	4	2.7	S
2(7)	×1(1)	10	9	6.4	F	×1(11)	11	1	0.4	S
2(8)	×1(1)	11	3	0.7	S	×1(11)	9	1	0.4	S
2(9)	×1(1)	11	0	0	S	×1(11)	8	8	9.1	F
2(10)	×1(1)	11	11	10.9	F	×1(11)	10	9	8.9	F
2(11)	×1(1)	10	0	0	S	×1(11)	11	10	10	F
2(12)	×1(1)	10	0	0	S	×1(11)	13	12	13.8	F
Family 3. Origin: 26664(35)×(31)										
3(1)	×1(1)	3	0	0	S	×2(7)	8	8	10.3	F
3(2)	×1(1)	7	0	0	S	×2(7)	4	2	3.8	F
3(3)	×1(1)	6	6	9.3	F	×2(7)	7	7	11	F

TABLE 6 (continued)

Family 4. Origin: 26502(7)×(6)															
4(1)	×1(1)	..	..	..	F	×1(11)	9	0	0	S					
4(2)	..	..	..	..	..	×1(11)	10	0	0	S					
4(3)	×1(3)	..	..	..	F	×1(11)	8	8	5.9	F					
4(4)	..	..	..	..	..	×1(11)	6	2	0.8	S					
4(5)	..	..	..	..	..	×1(11)	10	0	0	S					
Family 5. Origin: 26502(6)×(7)															
5(1)	×2(12)	7	7	15.6	F	×4(5)	8	8	10.6	F	×2(10)	9	0	0	S
5(2)	×2(12)	6	4	10.3	F	×4(5)	6	6	9.1	F	×2(10)	3	0	0	S
5(3)	×2(12)	9	9	11.8	F	×4(5)	9	0	0	S	×2(10)	10	10	7.6	F
5(4)	×2(12)	7	6	6.9	F	×4(5)	7	4	1.4	S	×2(10)	7	7	13.9	F
5(5)	×2(12)	10	10	15.4	F	×4(5)	6	6	13.3	F	×2(10)	7	0	0	S
5(6)	×2(12)	10	10	13.4	F	×4(5)	8	8	13.5	F	×2(10)	8	0	0	S
Family 6. Origin: 26321(2)×(1)															
6(1)	×1(1)	3	3	8.7	F	×1(2)	8	8	7.8	F	..	..	..	..	..
6(2)	×1(1)	6	6	7.3	F	×1(2)	11	0	0	S	..	..	..	..	..
6(3)	×1(1)	8	8	6	F	×1(2)	7	7	6.7	F	×6(5)	6	0	0	S
6(4)	×1(1)	11	11	8.8	F	×1(2)	8	0	0	S	..	..	..	..	..
6(5)	×1(1)	6	6	8.8	F	×1(2)	8	8	7	F	..	..	..	..	..

TABLE 7

Seed counts of intra-family crosses made in the years 1929–30 and 1930–31. Families 7 through 59.

Family 7. Origin: 2(2)×1(1)										
7(1)	×7(1)	47	0	0	S	..	..	..	..	..
7(2)	×7(1)	9	0	0	S	×7(3)	4	3	5.9	F
7(3)	×7(1)	10	10	9	F	×7(3)	61	0	0	S
7(4)	×7(1)	5	5	8.4	F	×7(3)	4	0	0	S
7(5)	×7(1)	5	2	3	?	..	..	..	..	..
7(6)	×7(1)	8	3	1.3	S	..	..	..	..	..
7(7)	×7(1)	..	..	..	S	×7(3)	3	3	6.3	F
7(8)	×7(1)	7	1	0	S	×7(3)	3	3	10	F
7(9)	×7(1)	10	10	12.4	F	×7(3)	8	0	0	S
Family 8. Origin: 2(10)×1(11)										
8(1)	×8(1)	21	0	0	S	×8(5)	7	7	13	F
8(2)	×8(1)	7	0	0	S	×8(5)	7	7	9	F
8(3)	×8(1)	8	0	0	S	×8(5)	6	6	9	F
8(4)	×8(1)	10	0	0	S	×8(5)	7	7	10	F
8(5)	×8(1)	5	5	14	F	×8(5)	36	0	0	S
8(6)	×8(1)	8	8	9	F	×8(5)	7	0	0	S
8(7)	×8(1)	5	5	10	F	×8(5)	8	0	0	S
8(8)	×8(1)	11	2	0.1	S	×8(5)	7	7	7.8	F
8(9)	×8(1)	9	0	0	S	..	..	..	..	..
8(10)	×8(1)	9	0	0	S	..	..	..	..	..

TABLE 7 (continued)

Family 9. Origin: 5(3)×2(12)															
9(1)	×9(1)	..	..	..	S	..	..	..	..	..	..	..	..	..	..
9(2)	×9(1)	12	12	10	F	×9(3)	19	4	1.9	S	×9(2)	41	0	0	S
9(3)	×9(1)	16	16	12	F	×9(3)	18	0	0	S	×9(2)	7	0	0	S
9(4)	×9(1)	8	0	0	S	×9(3)	9	9	12	F	..	..	..	..	..
9(5)	×9(1)	11	3	0.3	S	×9(3)	9	9	14	F	..	..	..	..	..
9(6)	×9(1)	9	0	0	S	×9(3)	11	11	11.5	F	..	..	..	..	..
9(7)	×9(1)	8	2	0.6	S	×9(3)	10	10	10	F	..	..	..	..	..
9(8)	×9(1)	9	9	11	F	..	..	..	..	..	×9(2)	11	3	2	S
9(9)	×9(1)	10	10	10	F	..	..	..	..	..	×9(2)	9	2	1	S
9(10)	×9(1)	12	0	0	S	..	..	..	..	..	×9(2)	12	9	10.5	F
9(11)	×9(1)	10	0	0	S	..	..	..	..	..	×9(2)	10	10	12.5	F
Family 10. Origin: 2(2)×1(2)															
10(1)	×10(1)	..	..	..	S										
10(2)	×10(1)	12	0	0	S										
10(3)	×10(1)	6	0	0	S										
10(4)	×10(1)	9	0	0	S										
10(5)	×10(1)	9	1	0.1	S										
10(6)	×10(1)	7	0	0	S										
10(7)	×10(1)	9	0	0	S										
10(8)	×10(1)	8	0	0	S										
10(9)	×10(1)	9	0	0	S										
10(10)	×10(1)	9	3	0.4	S										
Family 11. Origin: 2(12)×1(11)															
11(1)	×11(1)	72	0	0	S										
11(2)	×11(1)	11	0	0	S										
11(3)	×11(1)	6	6	6	F										
11(4)	×11(1)	10	1	0.2	S										
11(5)	×11(1)	9	1	0.1	S										
11(6)	×11(1)	10	10	8.4	F										
11(7)	×11(1)	10	3	1.4	S										
11(8)	×11(1)	9	9	14	F										
11(9)	×11(1)	8	0	0	S										
11(10)	×11(1)	5	5	11.2	F										
Family 12. Origin: 2(10)×1(1)															
12(1)	×12(1)	27	0	0	S										
12(2)	×12(1)	9	0	0	S										
12(3)	×12(1)	6	1	0.3	S										
12(4)	×12(1)	7	1	0.1	S										
12(5)	×12(1)	12	0	0	S										
12(6)	×12(1)	9	0	0	S										
12(7)	×12(1)	11	11	11.7	F										
12(8)	×12(1)	9	2	1	S										
12(9)	×12(1)	11	11	12	F										
12(10)	×12(1)	7	7	10.4	F										
12(11)	×12(1)	9	1	0.1	S										

TABLE 7 (continued)

Family 13. Origin: 5(3)×2(10)										
13(1)	×13(1)	47	0	0	S					
13(2)	×13(1)	8	0	0	S					
13(3)	×13(1)	9	0	0	S					
13(4)	×13(1)	6	6	9	F					
13(5)	×13(1)	11	0	0	S					
13(6)	×13(1)	7	0	0	S					
13(7)	×13(1)	10	0	0	S					
13(8)	×13(1)	7	7	9.4	F					
13(9)	×13(1)	8	0	0	S					
Family 14. Origin: 2(4)×1(2)										
14(1)	×14(1)	26	0	0	S	×14(2)	6	6	12.8	F
14(2)	×14(1)	10	10	9.8	F	×14(2)	31	0	0	S
14(3)	×14(1)	9	9	10.3	F	×14(2)	6	0	0	S
14(4)	×14(1)	6	0	0	S	..	..	..	..	..
14(5)	×14(1)	10	10	10.9	F	×14(2)	7	0	0	S
14(6)	×14(1)	6	0	0	S	×14(2)	6	6	12.3	F
14(7)	×14(1)	8	8	11.9	F	×14(2)	8	0	0	S
14(8)	×14(1)	7	0	0	S	×14(2)	6	6	13.7	F
14(9)	×14(1)	7	0	0	S	×14(2)	6	6	10.3	F
14(10)	×14(1)	6	6	8.2	F	×14(2)	10	0	0	S
14(11)	×14(1)	6	4	8.3	F	×14(2)	7	0	0	S
14(12)	×14(1)	9	0	0	S	..	..	..	..	..
14(13)	×14(1)	8	0	0	S	..	..	..	..	..
14(14)	×14(1)	7	0	0	S	..	..	..	..	..
Family 15. Origin: 5(6)×4(5)										
15(1)	×15(1)	41	0	0	S					
15(2)	×15(1)	7	7	10.7	F					
15(3)	×15(1)	10	10	12.5	F					
15(4)	×15(1)	8	0	0	S					
15(5)	×15(1)	11	0	0	S					
15(6)	×15(1)	7	3	0.4	S					
15(7)	×15(1)	9	0	0	S					
15(8)	×15(1)	9	1	0.3	S					
15(9)	×15(1)	9	4	1.3	S					
15(10)	×15(1)	9	0	0	S					
Family 16. Origin: 2(11)×1(11)										
16(1)	×16(1)	..	..	..	S					
16(2)	×16(1)	7	0	0	S					
16(3)	×16(1)	7	0	0	S					
16(4)	×16(1)	9	9	9.9	F					
16(5)	×16(1)	5	5	11.6	F					
16(6)	×16(1)	8	8	10.8	F					
16(7)	×16(1)	5	0	0	S					

TABLE 7 (continued)

Family 17. Origin: 5(5)×2(12)																
17(1)	×17(1)	24	0	0	S	..	..	..	..	..						
17(2)	×17(1)	8	7	9.6	F	..	..	..	..	..						
17(3)	×17(1)	8	3	0.4	S	×17(5)	8	0	0	S						
17(4)	×17(1)	6	2	0.8	S	×17(5)	7	0	0	S						
17(5)	×17(1)	6	6	4	F	×17(5)	200	8	..	S						
17(6)	×17(1)	7	4	3.4	F	×17(5)	6	1	1.2	S						
17(7)	×17(1)	8	7	7.1	F	×17(5)	6	2	0.2	S						
17(8)	×17(1)	7	3	1.6	S	×17(5)	10	4	1.2	S						
17(9)	×17(1)	7	0	0	S	×17(5)	6	0	0	S						
17(10)	×17(1)	9	7	6.5	F	×17(5)	6	6	12.3	F						
Family 18. Origin: 5(6)×2(12)																
18(1)	×18(1)	71	0	0	S	..	..	..	..	..	..	..	..	..	..	
18(2)	×18(1)	6	6	13.3	F	..	..	..	..	..	..	..	..	.	..	
18(3)	×18(1)	7	7	15	F	×18(3)	..	..	..	S	×18(4)	7	6	5.4	F	
18(4)	×18(1)	6	0	0	S	..	..	..	..	..	..	..	..	..	..	
18(5)	×18(1)	8	0	0	S	..	..	..	..	..	..	..	..	..	..	
18(6)	×18(1)	8	8	12.3	F	..	..	..	..	..	..	..	..	..	..	
18(7)	×18(1)	8	8	9.4	F	×18(3)	7	0	0	S	..	..	..	..	..	
18(8)	×18(1)	5	2	0.6	S	×18(3)	8	0	0	S	..	..	..	..	..	
18(9)	×18(1)	6	6	11.7	F	×18(3)	8	0	0	S	..	..	..	..	..	
18(10)	×18(1)	6	0	0	S	×18(3)	7	5	5.9	F	..	..	..	..	..	
Family 19. Origin: 2(1)×1(2)																
19(2)	×19(2)	..	..	..	S											
19(3)	×19(2)	6	0	0	S											
19(5)	×19(2)	5	0	0	S											
19(6)	×19(2)	5	5	9.8	F											
19(7)	×19(2)	7	0	0	S											
Family 20. Origin: 2(3)×1(2)																
20(1)	×20(1)	..	..	..	S											
20(2)	×20(1)	8	0	0	S											
20(3)	×20(1)	6	0	0	S											
20(4)	×20(1)	9	1	0.1	S											
20(5)	×20(1)	7	7	8	F											
20(6)	×20(1)	7	7	10.7	F											
20(7)	×20(1)	8	8	13.6	F											
20(8)	×20(1)	6	1	0.3	S											
20(9)	×20(1)	7	7	15.7	F											
20(10)	×20(1)	6	0	0	S											

TABLE 7 (continued)

Family 25. Origin: 2(7)×1(1)										
25(1)	×25(1)	..	..	..	S					
25(2)	×25(1)	6	0	0	S					
25(3)	×25(1)	7	7	8.3	F					
25(4)	×25(1)	8	8	9.3	F					
25(5)	×25(1)	6	1	0.2	S					
25(6)	×25(1)	8	0	0	S					
25(7)	×25(1)	8	1	0.1	S					
25(8)	×25(1)	8	8	8	F					
25(9)	×25(1)	8	0	0	S					
Family 26. Origin: 2(9)×1(11)										
26(1)	×26(1)	..	..	..	S					
26(2)	×26(1)	7	7	11.7	F					
26(3)	×26(1)	6	0	0	S					
26(4)	×26(1)	5	5	11.2	F					
26(5)	×26(1)	6	0	0	S					
26(6)	×26(1)	7	7	13.7	F					
26(7)	×26(1)	8	8	8	F					
26(8)	×26(1)	8	2	0.8	S					
26(9)	×26(1)	..	..	..	F					
26(10)	×26(1)	8	0	0	S					
Family 27. Origin: 3(1)×2(7)										
27(1)	×27(1)	..	..	..	S					
27(2)	×27(1)	7	0	0	S					
27(3)	×27(1)	6	0	0	S					
27(4)	×27(1)	7	7	6.1	F					
27(5)	×27(1)	5	0	0	S					
27(6)	×27(1)	6	6	7.8	F					
27(7)	×27(1)	7	7	6.3	F					
27(8)	×27(1)	5	5	8.4	F					
27(9)	×27(1)	6	6	5.5	F					
27(10)	×27(1)	2	1	1	S					
Family 29. Origin: 3(3)×1(1)										
29(1)	×29(1)	50	0	0	S	×29(2)	6	6	12	F
29(2)	×29(1)	5	5	9.8	F	×29(2)	38	0	0	S
29(3)	×29(1)	9	9	11.3	F	×29(2)	7	0	0	S
29(4)	×29(1)	5	5	7	F	×29(2)	6	0	0	S
29(5)	×29(1)	8	0	0	S	×29(2)	7	7	9.7	F
29(6)	×29(1)	9	9	8.4	F	×29(2)	8	0	0	S
29(7)	×29(1)	8	8	7.8	F	×29(2)	7	1	1	S
29(8)	×29(1)	7	0	0	S	×29(2)	7	7	10.3	F
29(9)	×29(1)	7	6	6.1	F	×29(2)	8	0	0	S
29(10)	×29(1)	6	0	0	S	×29(2)	7	7	9.9	F

TABLE 7 (continued)

Family 30. Origin: 3(3)×2(7)					
30(1)	×30(1)	..	..	..	S
30(2)	×30(1)	6	0	0	S
30(3)	×30(1)	7	0	0	S
30(4)	×30(1)	6	0	0	S
30(5)	×30(1)	6	0	0	S
30(6)	×30(1)	6	0	0	S
30(7)	×30(1)	5	0	0	S
30(8)	×30(1)	8	0	0	S
30(9)	×30(1)	5	0	0	S
30(10)	×30(1)	7	0	0	S
Family 31. Origin: 4(3)×1(11)					
31(1)	×31(1)	..	..	..	S
31(2)	×31(1)	6	0	0	S
31(3)	×31(1)	10	10	12.7	F
31(4)	×31(1)	6	6	9	F
31(5)	×31(1)	7	0	0	S
31(6)	×31(1)	8	0	0	S
31(7)	×31(1)	8	0	0	S
31(8)	×31(1)	6	6	14.3	F
31(9)	×31(1)	6	0	0	S
31(10)	×31(1)	7	0	0	S
Family 32. Origin: 5(1)×2(12)					
32(1)	×32(1)	..	..	..	S
32(2)	×32(1)	7	7	10.7	F
32(3)	×32(1)	8	8	10.5	F
32(4)	×32(1)	7	0	0	S
32(5)	×32(1)	8	8	11	F
32(6)	×32(1)	5	5	9.2	F
32(7)	×32(1)	9	0	0	S
32(8)	×32(1)	8	0	0	S
32(9)	×32(1)	8	0	0	S
32(10)	×32(1)	7	7	7.3	F
Family 33. Origin: 5(1)×4(5)					
33(1)	×33(2)	9	9	14.9	F
33(2)	×33(2)	..	..	..	S
33(3)	×33(2)	9	0	0	S
33(4)	×33(2)	6	6	7.8	F
33(5)	×33(2)	8	8	14.4	F
33(6)	×33(2)	7	7	8.7	F
33(7)	×33(2)	6	0	0	S
33(8)	×33(2)	6	1	1.5	S
33(9)	×33(2)	8	8	14.8	F
33(10)	×33(2)	7	7	10.4	F

TABLE 7 (continued)

Family 34. Origin: 8(1)×8(5)										
34(1)	×34(1)	109	0	0	S	×34(3)	4	4	9.3	F
34(2)	×34(1)	6	0	0	S	×34(3)	5	5	6.2	F
34(3)	×34(1)	9	9	9.4	F	×34(3)	..	..	..	S
34(4)	×34(1)	6	0	0	S	×34(3)	8	8	10.3	F
34(5)	×34(1)	5	5	14.8	F	×34(3)	6	0	0	S
34(6)	×34(1)	7	2	2.1	S	×34(3)	6	6	6.7	F
34(7)	×34(1)	8	3	2.9	F	×34(3)	6	6	6.5	F
34(8)	×34(1)	7	7	8	F	×34(3)	7	0	0	S
34(9)	×34(1)	8	8	7.8	F	×34(3)	8	0	0	S
34(10)	×34(1)	6	0	0	S	×34(3)	7	0	0	S
Family 35. Origin: 8(5)×8(1)										
35(1)	×35(1)	..	..	..	S	×35(5)	8	8	7	F
35(2)	×35(1)	10	0	0	S	×35(5)	7	7	9.7	F
35(3)	×35(1)	7	0	0	S	×35(5)	8	8	7	F
35(4)	×35(1)	7	7	8.9	F	×35(5)	7	0	0	S
35(5)	×35(1)	10	10	10.1	F	×35(5)	..	..	..	S
35(6)	×35(1)	7	7	9.7	F	×35(5)	7	0	0	S
35(7)	×35(1)	7	0	0	S	×35(5)	6	6	5.8	F
35(8)	×35(1)	6	6	7	F	×35(5)	7	0	0	S
35(9)	×35(1)	6	0	0	S	×35(5)	5	5	5.4	F
35(10)	×35(1)	6	0	0	S	×35(5)	6	6	5.3	F
Family 36. Origin: 9(2)×12(1)										
36(1)	×36(1)	..	..	..	S	×36(4)	6	6	9.8	F
36(2)	×36(1)	9	0	0	S	×36(4)	7	7	5.7	F
36(3)	×36(1)	6	0	0	S	×36(4)	8	8	14.7	F
36(4)	×36(1)	11	11	5.5	F	×36(4)	..	..	..	S
36(5)	×36(1)	12	1	0	S	×36(4)	9	9	6.9	F
36(6)	×36(1)	6	6	5.7	F	×36(4)	8	0	0	S
36(7)	×36(1)	7	7	9.3	F	×36(4)	6	0	0	S
36(8)	×36(1)	7	0	0	S	×36(4)	7	7	8.4	F
36(9)	×36(1)	7	0	0	S	×36(4)	6	5	4.8	F
36(10)	×36(1)	7	7	3.9	F	×36(4)	7	0	0	S
Family 37. Origin: 12(1)×9(2)										
37(1)	×37(1)	64	0	0	S	×37(2)	6	6	7.2	F
37(2)	×37(1)	7	7	6.3	F	×37(2)	..	..	..	S
37(3)	×37(1)	7	7	7.1	F	×37(2)	7	0	0	S
37(4)	×37(1)	7	0	0	S	×37(2)	6	6	6.8	F
37(5)	×37(1)	10	0	0	S	×37(2)	6	6	7.2	F
37(6)	×37(1)	6	0	0	S	×37(2)	6	6	9.8	F
37(7)	×37(1)	7	0	0	S	×37(2)	6	6	5.3	F
37(8)	×37(1)	7	7	10.4	F	×37(2)	6	0	0	S
37(9)	×37(1)	6	5	6.3	F	×37(2)	6	0	0	S
37(10)	×37(1)	5	5	9.4	F	×37(2)	6	0	0	S

TABLE 7 (continued)

Family 38. Origin: 9(6)×10(1)										
38(1)	×38(1)	63	0	0	S	×38(6)	5	5	7	F
38(2)	×38(1)	7	0	0	S	×38(6)	7	0	0	S
38(3)	×38(1)	8	0	0	S	×38(6)	5	5	5	F
38(4)	×38(1)	8	8	9.3	F	×38(6)	8	1	0.4	S
38(5)	×38(1)	7	5	6.1	F	×38(6)	7	0	0	S
38(6)	×38(1)	8	8	8.3	F	×38(6)	67	0	0	S
38(7)	×38(1)	8	1	0.3	S	×38(6)	5	5	3.4	F
38(8)	×38(1)	8	2	0.6	S	×38(6)	5	4	3	F
38(9)	×38(1)	7	7	9.6	F	×38(6)	6	4	5.8	F
38(10)	×38(1)	6	0	0	S	×38(6)	6	6	10	F
Family 39. Origin: 10(1)×9(1)										
39(1)	×39(1)	..	..	..	S	×39(5)	6	6	7.8	F
39(2)	×39(1)	12	0	0	S	×39(5)	6	6	8.2	F
39(3)	×39(1)	18	3	0.3	S	×39(5)	10	10	11.4	F
39(4)	×39(1)	10	0	0	S	×39(5)	8	8	8.4	F
39(5)	×39(1)	7	7	9.6	F	×39(5)	..	..	..	S
39(6)	×39(1)	8	0	0	S	×39(5)	7	7	8.9	F
39(7)	×39(1)	9	0	0	S	×39(5)	6	6	10.5	F
39(8)	×39(1)	8	1	0.3	S	×39(5)	8	8	8.4	F
39(9)	×39(1)	18	16	8.5	F	×39(5)	14	14	9.6	F
39(10)	×39(1)	8	0	0	S	×39(5)	8	8	7	F
Family 42. Origin: 8(5)×9(1)										
42(1)	×42(1)	..	..	..	S	×42(4)	6	6	10	F
42(2)	×42(1)	7	7	10.3	F	×42(4)	8	0	0	S
42(3)	×42(1)	9	9	9.2	F	×42(4)	7	0	0	S
42(4)	×42(1)	8	8	9.8	F	×42(4)	..	..	..	S
42(5)	×42(1)	10	10	8.2	F	×42(4)	7	0	0	S
42(6)	×42(1)	7	1	0.4	S	×42(4)	5	3	2.8	F
42(7)	×42(1)	7	7	8.9	F	×42(4)	7	0	0	S
42(8)	×42(1)	8	8	12.9	F	×42(4)	7	0	0	S
42(9)	×42(1)	8	0	0	S	×42(4)	5	5	7.8	F
42(10)	×42(1)	6	0	0	S	×42(4)	6	6	6.3	F
..	..	..	..	..	..	..				
..	..	..	..	..	..	..				
×42(3)	101	0	0	S						
..	..	..	..	..						
..	..	..	..	..						
..	..	..	..	..						
..	..	..	..	..						
..	..	..	..	..						
..	..	..	..	..						
Family 43. Origin: 8(5)×14(9)										
43(1)	×43(1)	..	..	..	S	×43(2)	7	7	9.8	F
43(2)	×43(1)	9	9	6.8	F	×43(2)	..	..	..	S
43(3)	×43(1)	7	0	0	S	×43(2)	7	7	6.6	F
43(4)	×43(1)	6	6	7.7	F	×43(2)	7	0	0	S
43(5)	×43(1)	7	6	8.8	F	×43(2)	6	2	0.3	S
43(6)	×43(1)	6	6	5.2	F	×43(2)	7	0	0	S
43(7)	×43(1)	7	7	10.9	F	×43(2)	7	0	0	S
43(8)	×43(1)	7	0	0	S	×43(2)	12	7	4.8	F
43(9)	×43(1)	6	6	12.5	F	×43(2)	7	0	0	S
43(10)	×43(1)	6	6	8	F	×43(2)	7	0	0	S

TABLE 7 (continued)

Family 44. Origin: 9(2)×9(1)										
44(1)	×44(1)	..	..	..	S	×44(10)	7	7	9	F
44(2)	×44(1)	7	0	0	S	×44(10)	6	6	7.2	F
44(3)	×44(1)	10	0	0	S	×44(10)	7	7	7.7	F
44(4)	×44(1)	6	0	0	S	×44(10)	7	7	10.3	F
44(5)	×44(1)	15	3	0.3	S	×44(10)	7	7	10	F
44(6)	×44(1)	7	1	0.3	S	×44(10)	6	6	8.5	F
44(7)	×44(1)	8	0	0	S	×44(10)	6	6	9.2	F
44(8)	×44(1)	8	0	0	S	×44(10)	7	7	9.6	F
44(9)	×44(1)	5	5	6.2	F	×44(10)	7	0	0	S
44(10)	×44(1)	5	5	6	F	×44(10)	..	..	..	S
44(11)	×44(1)	6	0	0	S	..	..	..	..	..
44(12)	×44(1)	7	0	0	S	..	..	..	..	..
Family 45. Origin: 9(3)×9(1)										
45(1)	×45(1)	..	..	..	S	×45(6)	6	6	7.5	F
45(2)	×45(1)	5	2	0.8	S	×45(6)	7	7	5.4	F
45(3)	×45(1)	10	10	6.2	F	×45(6)	8	1	0.1	S
45(4)	×45(1)	8	0	0	S	×45(6)	7	7	6.1	F
45(5)	×45(1)	7	7	4	F	×45(6)	7	1	0.1	S
45(6)	×45(1)	9	9	8.4	F	×45(6)	..	..	..	S
45(7)	×45(1)	8	8	8.3	F	×45(6)	8	0	0	S
45(8)	×45(1)	8	8	11.1	F	×45(6)	9	0	0	S
45(9)	×45(1)	8	3	0.4	S	×45(6)	8	8	10.6	F
45(10)	×45(1)	7	1	1.4	S	×45(6)	6	6	6.5	F
Family 49. Origin: 5(3)×2(12)										
49(1)	×49(1)	43	1	..	S	×49(6)	5	5	8	F
49(2)	×49(1)	9	0	0	S	×49(6)	7	7	10.1	F
49(3)	×49(1)	7	0	0	S	×49(6)	7	7	8.1	F
49(4)	×49(1)	8	0	0	S	×49(6)	5	5	8.4	F
49(5)	×49(1)	6	4	1.8	S	×49(6)	8	8	8.3	F
49(6)	×49(1)	10	9	5.4	F	×49(6)	..	..	..	S
49(7)	×49(1)	10	9	8.9	F	×49(6)	7	0	0	S
49)8)	×49(1)	7	7	6.3	F	×49(6)	7	0	0	S
49(9)	×49(1)	11	11	9.7	F	×49(6)	6	0	0	S
49(10)	×49(1)	8	0	0	S	×49(6)	7	7	6.1	F
Family 51. Origin: 7(4)×7(1)										
51(1)	×51(1)	..	..	..	S	×51(5)	6	6	9.8	F
51(2)	×51(1)	6	0	0	S	×51(5)	5	5	6.2	F
51(3)	×51(1)	8	5	3	F	×51(5)	5	1	0.4	S
51(4)	×51(1)	7	1	0.1	S	×51(5)	6	6	5.7	F
51(5)	×51(1)	8	8	5.3	F	×51(5)	..	..	..	S
51(6)	×51(1)	7	0	0	S	×51(5)	6	6	10.7	F
51(7)	×51(1)	4	4	3	F	×51(5)	7	0	0	S
51(8)	×51(1)	6	0	0	S	×51(5)	6	6	6.8	F

TABLE 7 (continued)

Family 52. Origin: 7(9)×7(1)										
52(1)	×52(1)	..	..	..	S	×52(2)	5	3	3.6	F
52(2)	×52(1)	10	10	10.7	F	×52(2)	..	..	..	S
52(3)	×52(1)	7	0	0	S	×52(2)	7	7	6.3	F
52(4)	×52(1)	9	3	0.6	S	×52(2)	4	4	7.3	F
52(5)	×52(1)	5	5	8	F	×52(2)	5	1	0.4	S
52(6)	×52(1)	7	7	7.1	F	×52(2)	7	0	0	S
52(7)	×52(1)	7	7	9.8	F	×52(2)	7	0	0	S
52(8)	×52(1)	6	6	9.7	F	×52(2)	6	1	0.2	S
52(9)	×52(1)	4	4	5.3	F	×52(2)	7	0	0	S
52(10)	×52(1)	7	7	6.1	F	×52(2)	6	0	0	S
Family 53. Origin: 2(2)×1(1)										
53(1)	×53(1)	..	..	..	S	×53(3)	6	6	7.2	F
53(2)	×53(1)	8	0	0	S	×53(3)	6	6	6	F
53(3)	×53(1)	6	6	6.7	F	×53(3)	..	..	..	S
53(4)	×53(1)	6	0	0	S	×53(3)	5	5	7.6	F
53(5)	×53(1)	4	1	0.3	S	×53(3)	6	0	0	S
53(6)	×53(1)	8	0	0	S	×53(3)	6	6	?	F
53(7)	×53(1)	6	0	0	S	×53(3)	6	6	8.8	F
53(8)	×53(1)	6	0	0	S	×53(3)	6	6	7	F
53(9)	×53(1)	6	6	5.3	F	×53(3)	6	0	0	S
53(10)	×53(1)	7	0	0	S	×53(3)	7	7	7.3	F
Family 56. Origin: 2(10)×1(11)										
56(1)	×56(1)	..	..	..	S	×56(3)	7	6	5.9	F
56(2)	×56(1)	7	7	10.9	F	×56(3)	7	0	0	S
56(3)	×56(1)	6	6	6.7	F	×56(3)	..	..	..	S
56(4)	×56(1)	8	0	0	S	×56(3)	6	6	6.7	F
56(5)	×56(1)	8	0	0	S	×56(3)	6	6	7.8	F
56(6)	×56(1)	8	0	0	S	×56(3)	6	6	8.7	F
56(7)	×56(1)	7	0	0	S	×56(3)	6	6	7.2	F
56(8)	×56(1)	7	0	0	S	×56(3)	6	6	11.8	F
56(9)	×56(1)	6	0	0	S	×56(3)	8	8	6.3	F
56(10)	×56(1)	9	9	5.8	F	×56(3)	8	1	0.3	S
Family 59. Origin: 10(1)×9(2)										
59(1)	×59(1)	..	..	..	S	×59(2)	6	6	10.3	F
59(2)	×59(1)	11	11	9.7	F	×59(2)	..	..	..	S
59(3)	×59(1)	10	1	0.1	S	×59(2)	7	7	9.1	F
59(4)	×59(1)	9	9	12.8	F	×59(2)	6	0	0	S
59(5)	×59(1)	9	9	6.3	F	×59(2)	7	0	0	S
59(6)	×59(1)	8	0	0	S	×59(2)	7	7	6.1	F
59(7)	×59(1)	6	2	1.2	S	×59(2)	7	7	9.9	F
59(8)	×59(1)	6	1	0.2	S	×59(2)	6	6	12.2	F
59(9)	×59(1)	7	0	0	S	×59(2)	8	8	10.3	F
59(10)	×59(1)	6	0	0	S	×59(2)	7	7	9.6	F

TABLE 8

Seed counts of inter-family crosses made in the year 1929–30. Families 7 through 33.

7(1)	×9(6)	6	3	2.2	S	×9(9)	5	5	7.6	F	..	..	..	..	..
7(3)	×9(6)	5	5	13	F	×9(9)	5	5	7	F	×14(9)	6	0	0	S
8(5)	×9(1)	6	6	12.8	F	×9(2)	8	0	0	S	×14(9)	6	6	11.6	F
8(5)	..	..	..	..	..	×9(9)	6	0	0	S	..	..	..	..	..
8(8)	..	..	..	..	..	×9(9)	6	6	10.3	F	×14(9)	5	1	1	S
8(10)	..	..	..	..	..	×9(2)	10	4	(inj)	F	..	..	..	..	..
9(1)	×12(1)	10	1	0.1	S	×12(7)	14	3	0.3	S	..	..	..	..	..
9(2)	×12(1)	8	8	11	F	..	..	..	..	..	..	..	..	..	..
9(3)	..	..	..	..	..	×12(7)	5	0	0	S	..	..	..	..	..
9(6)	×10(1)	8	8	9.6	F	..	..	..	..	..	..	..	..	..	..
9(8)	×10(1)	6	1	(old)	?	..	..	..	..	..	..	..	..	..	..
9(9)	×12(9)	6	0	0	S	..	..	..	..	..	..	..	..	..	.
10(1)	×9(1)	6	6	12.7	F	×9(2)	7	7	12.3	F	×14(9)	8	1	0.1	S
11(2)	×9(1)	9	1	1	S	×9(9)	6	6	8.7	F	×14(9)	4	4	9.1	F
11(2)	×9(6)	9	0	0.1	S	..	..	..	..	..	..	..	..	..	..
11(6)	×9(1)	6	6	11	F	×9(9)	7	0	0	S	×14(9)	5	5	8.8	F
12(1)	×9(1)	8	2	0.5	S	×9(2)	7	7	10.5	F	..	..	..	..	..
12(9)	..	..	..	..	..	×9(3)	8	8	13.5	F	..	..	..	..	..
12(10)	×9(6)	6	5	9.8	F	×9(9)	5	3	2.8	F	..	..	..	..	..
13(3)	..	..	..	..	..	×9(3)	7	0	0	S	..	..	..	..	..
13(4)	..	..	..	..	..	×9(3)	6	6	11	F	..	..	..	..	..
13(8)	..	..	..	..	..	×9(6)	5	5	11.8	F	×14(9)	6	0	0	S
14(3)	×9(1)	6	6	10.5	F	×9(3)	6	0	0	S	..	..	..	..	..
14(4)	×9(1)	6	6	12	F	×9(3)	7	7	10.1	F	..	..	..	..	..
14(9)	×10(1)	5	0	0	S	..	..	..	..	..	..	..	..	..	..
15(8)	×9(6)	9	9	14.1	F	×9(9)	7	7	14.7	F	×14(9)	6	2	0.5	S
15(2)	×9(6)	..	..	..	F	×9(9)	..	..	..	S	..	..	..	..	..
16(1)	..	..	..	..	..	×9(9)	5	5	9.8	F	..	..	..	..	..
17(3)	×9(6)	7	0	0	S	×9(3)	7	7	10	F	×14(9)	6	6	3	F
18(9)	..	..	..	..	..	×9(9)	7	7	10.3	F	..	..	..	..	..
20(9)	×19(2)	6	6	8.8	F	..	..	..	..	..	..	..	..	..	..
20(10)	×19(2)	6	0	0	S	..	..	..	..	..	..	..	..	..	..
29(7)	×9(6)	6	0	0	S	×9(9)	6	6	8	F	..	..	..	..	..
29(8)	×9(6)	6	6	8	F	×9(9)	6	0	0	S*	×14(9)	8	0	0	S*
30(5)	×9(6)	7	6	7.3	F	×9(9)	..	..	..	F	×14(9)	7	0	0	S
30(7)	×32(1)	5	5	11.2	F	×32(2)	6	0	0	S	..	..	..	..	..
30(10)	×32(1)	5	5	10.8	F	×32(2)	..	..	..	S	..	..	..	..	..
31(1)	×9(6)	..	..	..	F	×9(9)	..	..	..	S	..	..	..	..	..
31(8)	×9(6)	7	7	5.4	F	×9(9)	8	6	4.5	F	..	..	..	..	..
31(4)	×32(2)	6	1	0.2	S	..	..	..	..	..	..	..	..	..	.
31(3)	×32(1)	4	4	10.8	F	..	..	..	..	..	..	..	..	..	..
32(1)	×9(6)	7	0	0	S	×9(9)	..	..	..	F	..	..	..	..	..
33(4)	×32(1)	6	6	12.7	F	×32(2)	6	0	0	S	×14(9)	6	0	0	S
33(7)	×32(1)	6	6	14.5	F	×32(2)	5	5	14.8	F	×9(9)	8	0	0	S

* These two crosses are anomalous. Fertility was expected in each case, and the peculiar results are probably due to some form of true sterility.

TABLE 9

Seed counts of inter-family crosses made in the year 1930–31 between several families and others which represent the sibs of their parents.

34(1)	×56(1)	6	6	8	F	×56(3)	7	0	0	S	..	..	..	..	..
34(3)	×56(1)	6	0	0	S	×56(3)	6	6	8.2	F	..	..	..	..	..
56(1)	×34(1)	7	7	13.1	F	×34(3)	7	0	0	S	..	..	..	..	..
56(2)	×34(1)	5	0	0	S	..	..	..	..	..	..	..	..	..	.
56(3)	×34(1)	7	0	0	S	×34(3)	8	8	5	F	..	..	..	..	..
35(2)	×56(2)	7	0	0	S	×56(1)	6	6	5.8	F	..	..	..	..	..
35(4)	×56(2)	6	6	8.3	F	×56(1)	7	0	0	S	..	..	..	..	..
56(2)	×35(1)	6	0	0	S	×35(5)	6	6	8	F	..	..	..	..	..
56(3)	×35(1)	7	0	0	S	×35(5)	8	8	6.8	F	..	..	..	..	..
56(1)	×35(1)	5	5	7.8	F	×35(5)	9	0	0	S	..	..	..	..	..
44(3)	×49(1)	6	6	1	S	×49(8)	7	7	7.6	F	..	..	..	..	..
44(9)	×49(1)	5	5	8.6	F	×49(8)	6	0	0	S	..	..	..	..	..
49(1)	×44(1)	7	1	1.9	S	×44(2)	6	0	0	S	×44(3)	8	0	0	S
49(2)	×44(1)	9	3	0.6	S	×44(2)	7	3	1	S	×44(3)	7	2	1	S
49(3)	×44(1)	7	2	2.3	S	×44(2)	6	1	0.7	S	×44(3)	6	0	0	S
49(4)	×44(1)	6	1	0.7	S	..	..	..	..	..	×44(3)	5	0	0	S
49(6)	×44(1)	6	6	8.7	F	×44(2)	3	3	13	F	×44(3)	7	7	9.9	F
49(7)	×44(1)	3	3	4.7	F	..	..	..	..	..	..	..	..	..	..
49(8)	×44(1)	6	6	11	F	×44(2)	6	6	9.9	F	×44(3)	6	6	8.8	F
49(9)	..	..	..	..	..	×44(2)	7	7	9.1	F	..	..	..	..	..
49(1)	×44(6)	8	0	0	S	×44(9)	7	7	11.1	F	×44(10)	7	7	9.4	F
49(2)	×44(6)	7	0	0	S	..	..	..	..	..	×44(10)	6	6	12	F
49(3)	×44(6)	7	0	0	S	..	..	..	..	..	..	..	..	..	..
49(4)	×44(6)	6	0	0	S	..	..	..	..	..	..	..	..	..	..
49(6)	×44(6)	4	4	11.8	F	..	..	..	..	..	..	..	..	..	..
49(8)	×44(6)	6	6	9.7	F	×44(9)	7	2	0.4	S	..	..	..	..	..
45(4)	×49(1)	9	0	0	S	×49(6)	6	6	7.2	F	..	..	..	..	..
45(5)	×49(1)	9	9	5.7	F	×49(6)	6	0	0	S	..	..	..	..	..
49(1)	×45(4)	7	0	0	S	×45(5)	7	7	10.1	F	..	..	..	..	..
49(6)	×45(4)	10	10	8.5	F	×45(5)	7	0	0	S	..	..	..	..	..
51(1)	×53(3)	6	0	0	S	×53(7)	7	7	6.6	F	..	..	..	..	..
51(5)	×53(3)	5	5	3.2	F	×53(7)	6	0	0	S	..	..	..	..	..
53(7)	×51(1)	6	5	5	F	×51(5)	7	0	0	S	..	..	..	..	..
53(3)	×51(1)	6	0	0	S	×51(5)	6	6	4.3	F	..	..	..	..	..
52(1)	×53(1)	8	1	0.3	S	×53(6)	7	0	0	S	×53(3)	8	8	7.8	F
52(2)	×53(1)	9	9	10.6	F	×53(6)	6	6	9	F	×53(3)	7	0	0	S
53(1)	×52(1)	8	5	4	?	×52(2)	6	2	1	?	×52(4)	7	3	0.4	S
53(2)	×52(1)	8	3	0.6	S	×52(2)	6	3	2.7	F	×52(4)	5	3	0.4	S
53(3)	×52(1)	8	8	13.3	F	×52(2)	7	0	0	S	×52(4)	6	6	14.5	F
53(4)	×52(1)	8	0	0	S	×52(2)	6	3	1	?	×52(4)	5	0	0	S
53(5)	×52(1)	7	7	15.9	F	..	..	..	..	..	×52(4)	3	3	9.7	F
53(6)	×52(1)	8	0	0	S	..	..	..	..	..	×52(4)	6	0	0	S
53(7)	×52(1)	7	0	0	S	..	..	..	..	..	×52(4)	6	0	0	S

TABLE 10

Seed counts of inter-family crosses of the year 1930–31 between families which are the offspring of reciprocal crosses.

34(1)	×35(1)	7	0	0	S	×35(5)	7	7	7.1	F	..	..	..	..	..
34(3)	×35(1)	7	7	9.3	F	×35(5)	7	0	0	S	..	..	..	..	..
35(1)	×34(1)	6	0	0	S	×34(3)	6	5	5.2	F	..	..	..	..	..
35(5)	×34(1)	6	6	4.8	F	×34(3)	7	0	0	S	..	..	..	..	..
36(1)	×37(1)	16	3	0.9	S	×37(2)	6	6	11.2	F	..	..	..	..	..
36(4)	×37(1)	6	6	10.8	F	×37(2)	6	0	0	S	..	..	..	..	..
37(1)	×36(1)	15	2	0.3	S	×36(4)	7	7	13.8	F	..	..	..	..	..
37(2)	×36(1)	8	8	9.8	F	×36(4)	7	0	0	S	..	..	..	..	..
38(1)	×39(1)	13	3	0.2	S	×39(5)	9	9	11	F	×39(9)	9	9	9.2	F
38(6)	×39(1)	7	7	10.1	F	×39(5)	5	5	9.4	F	×39(9)	7	0	0	S
39(1)	×38(1)	7	0	0	S	×38(6)	6	6	9	F	..	..	..	..	..
39(5)	×38(1)	10	10	8.8	F	×38(6)	7	6	10.5	F	..	..	..	..	..
39(9)	×38(1)	9	9	8.1	F	×38(6)	8	0	0	S	..	..	..	..	..

TABLE 11

Seed counts of inter-family crosses chiefly of 1930–31, between families which had a common mother and different fathers.

35(2)	×43(1)	5	3	3	F	×43(2)	6	1	0.2	S
35(4)	×43(1)	7	0	0	S	×43(2)	6	6	8.7	F
43(1)	×35(2)	7	7	10.4	F	×35(4)	7	0	0	S
43(2)	×35(2)	7	0	0	S	×35(4)	6	6	11.2	F
36(3)	×44(1)	6	0	0	S	×44(9)	6	6	10.3	F
36(6)	×44(1)	6	6	10.2	F	×44(9)	6	0	0	S
44(1)	×36(1)	9	0	0	S	×36(6)	7	7	11.1	F
44(9)	×36(3)	6	6	9	F	×36(6)	6	0	0	S
35(1)	×42(1)	5	0	0	S	×42(3)	6	6	10.7	F
35(6)	×42(1)	4	4	7.5	F	×42(3)	4	4	6.5	F
42(1)	×35(1)	7	0	0	S	×35(6)	6	6	9.2	F
42(3)	×35(1)	5	4	2.8	F	×35(6)	7	7	7.4	F
42(4)	×43(2)	5	4	7.8	F	×43(1)	5	5	9.4	F
42(6)	×43(2)	7	0	0	S	×43(1)	8	8	4.1	F
43(1)	×42(4)	7	7	11.4	F	×42(6)	5	4	6.8	F
43(2)	×42(4)	6	6	12.8	F	×42(6)	7	2	0.1	S
39(4)	×59(3)	8	8	12.8	F	×59(2)	6	6	8.5	F
39(5)	×59(3)	7	0	0	S	×59(2)	7	7	8.1	F
59(3)	×39(4)	9	9	6.2	F	×39(5)	6	0	0	S
59(2)	×39(4)	7	7	9.7	F	×39(5)	7	6	8.6	F
33(4)	×32(1)	6	6	12.7	F	×32(2)	6	0	0	S
33(7)	×32(1)	6	6	14.5	F	×32(2)	5	5	14.8	F

TABLE 12

Seed counts of inter-family crosses of 1930–31 between families which had a common father but different mothers.

42(5)	×44(8)	6	0	0	S	×44(10)	8	8	6.1	F	..	..	..	..	..
42(6)	×44(8)	14	7	3.8	F	×44(10)	8	0	0	S	..	..	..	..	..
44(8)	×42(5)	7	1	0.1	S	×42(6)	5	4	3.6	F	..	..	..	.	
44(10)	×42(5)	5	4	3.2	F	×42(6)	6	0	0	S	..	..	..	..	..
42(1)	×45(4)	6	6	8.3	F	×45(5)	8	1	0.4	S	..	..	..	..	..
42(3)	×45(4)	6	0	0	S	×45(5)	7	5	5.4	F	..	..	..	..	..
45(4)	×42(1)	6	6	13.7	F	×42(3)	6	0	0	S	..	..	..	..	..
45(5)	×42(1)	7	0	0	S	×42(3)	7	7	5.3	F	..	..	..	.	..
44(6)	×45(1)	9	0	0	S	×45(6)	7	7	7.1	F	..	..	..	..	..
44(10)	×45(1)	8	8	10.4	F	×45(6)	7	0	0	S	..	..	..	..	..
45(1)	×44(6)	7	0	0	S	×44(10)	6	6	7.2	F	..	..	..	..	..
45(6)	×44(6)	7	7	8.3	F	×44(10)	7	1	0.4	S	×44(9)	7	0	0	S
51(1)	×52(3)	6	6	6.8	F	×52(6)	7	0	0	S	..	..	..	..	..
51(5)	×52(3)	6	0	0	S	×52(6)	6	6	8	F	..	..	..	..	..
52(3)	×51(1)	7	6	7.3	F	×51(5)	7	0	0	S	..	..	..	..	..
52(6)	×51(1)	7	0	0	S	×51(5)	7	7	8	F	..	..	..	..	..
39(2)	×44(2)	6	0	0	S	×44(10)	6	6	7.8	F	..	..	..	.	..
39(5)	×44(2)	6	6	10.8	F	×44(10)	6	0	0	S	..	..	..		..
44(2)	×39(1)	7	0	0	S	×39(5)	6	6	6.5	F	..	..	..	..	..
44(10)	×39(1)	7	6	6.4	F	×39(5)	7	0	0	S	..	..	..	..	..
39(2)	×45(4)	7	1	1.3	S	×45(6)	6	6	8.7	F	..	..	..	..	..
39(5)	×45(4)	7	7	6	F	×45(6)	7	0	0	S	..	..	..	..	..
45(4)	×39(2)	6	0	0	S	×39(5)	7	7	10.1	F	..	..	..	..	..
45(6)	×39(2)	6	6	8.7	F	×39(5)	5	0	0	S	..	..	..	..	..
37(1)	×59(1)	8	8	9.5	F	×59(4)	7	7	10.6	F	..	..	..	..	..
37(2)	×59(1)	7	1	0.3	S	×59(4)	7	7	7.9	F	..	..	..	..	..
59(1)	×37(1)	9	9	10.2	F	×37(2)	7	2	0.4	S	..	..	..	..	..
59(4)	×37(1)	6	6	12	F	×37(2)	4	4	10.8	F	..	..	..	..	..
39(6)	×42(5)	7	0	0.6	S	×42(1)	7	7	9.9	F	..	..	..	..	..
39(5)	×42(5)	5	5	2.8	F	×42(1)	6	0	0	S	..	..	..	..	..
42(5)	×39(6)	7	0	0	S	×39(5)	5	4	4.4	F	..	..	..	..	..
42(1)	×39(6)	7	7	7.7	F	×39(5)	6	0	0	S	..	..	..	..	..

TABLE 13

F_2 ratios from crosses between C. grandiflora and self-fertile species.

FAMILY	ORIGIN	RATIO	EXPECTED 3:1	EXPECTED 2:1
v2	v1(2)	39F:15S	41F:13S	36F:18S
v3	v1(6)	85F:39S	93F:31S	83F:41S
v5	v1(13)	69F:27S	72F:24S	64F:32S
r6	r3(4)	91F:32S	92F:31S	82F:41S
r7	r3(2)	31F:9S	30F:10S	27F:13S
r8	r3(5)	5F:2S	5F:2S	5F:2S
r12	r3(3)	39F:5S	33F:11S	29F:15S
r13	r4(1)	75F:25S	75F:25S	67F:33S
t5	t4(4)	31F:11S	32F:10S	28F:14S
t8	t1(1)	50F:25S	56F:19S	50F:25S
t10	t4(2)	34F:16S	38F:12S	33F:17S
t11	t4(5)	32F:18S	38F:12S	33F:17S
t12	t1(3)	56F:18S	56F:18S	49F:25S
t13	t1(2)	32F:18S	39F:13S	35F:17S
t21	t4(3)	36F:14S	38F:12S	33F:17S
t22	t4(6)	27F:9S	29F:9S	24F:12S
t23	t4(8)	30F:13S	32F:11S	29F:14S

TABLE 14

F_3 ratios from crosses between C. grandiflora and self-fertile species.

FAMILY	ORIGIN	RATIO	FAMILY	ORIGIN	RATIO
v6	v5(59)	60F:23S	r14	r7(7)	0F:2S
v7	v5(60)	86F:31S	r15	r7(11)	109F:15S
v8	v5(62)	122F:0S	r16	r7(12)	90F:35S
v9	v5(67)	122F:0S	r17	r7(15)	92F:32S
v10	v5(74)	31F:10S	r18	r7(18)	70F:30S
v11	v5(79)	124F:0S	r19	r7(20)	110F:0S
v12	v5(80)	123F:0S	r20	r7(36)	98F:25S
v13	v5(83)	86F:39S			
v14	v5(87)	76F:28S			
v15	v5(96)	98F:27S			

TABLE 15

Crosses between self-sterile segregates and their self-fertile and self-sterile sibs.

v2(1) (S)	×v2(5) (S)	8	0	0	S	v2(16) (S)	×v2(5) (S)	5	1	0.8	S
v2(9) (S)	×v2(5) (S)	6	3	0.2	S	v2(18) (S)	×v2(5) (S)	6	0	0	S
v2(13) (S)	×v2(5) (S)	8	1	0.4	S	v2(19) (S)	×v2(5) (S)	5	5	1.6	S
v2(14) (S)	×v2(5) (S)	9	3	0.5	S	v2(31) (F)	×v2(5) (S)	..	..	..	F
v2(15) (S)	×v2(5) (S)	6	3	0.8	S	v2(29) (F)	×v2(5) (S)	..	..	..	F
v3(2) (S)	×v3(1) (S)	6	0	0	S	v3(3) (S)	×v3(1) (S)	7	0	0	S
v3(55) (S)	×v3(1) (S)	5	1	0.2	S	v3(63) (S)	×v3(1) (S)	4	4	0	S
v3(72) (S)	×v3(1) (S)	7	0	0	S	v3(71) (S)	×v3(1) (S)	6	0	0	S
r6(40) (S)	×r6(51) (S)	6	1	0.2	S	r6(47) (S)	×r6(51) (S)	6	3	2	S
r6(41) (S)	×r6(51) (S)	7	7	0	S	r6(52) (S)	×r6(51) (S)	7	1	0.1	S
r6(68) (S)	×r6(51) (S)	6	2	0.3	S	r6(67) (S)	×r6(51) (S)	6	5	0.5	S
r6(16) (S)	×r6(51) (S)	6	0	0	S	r6(59) (S)	×r6(51) (S)	6	6	0	S
r6(44) (F)	×r6(51) (S)	5	4	1.2	S	r6(4) (F)	×r6(51) (S)	4	1	1.3	S
r6(55) (F)	×r6(51) (S)	5	2	2.2	S	r6(56) (F)	×r6(51) (S)	4	2	0.5	S
r13(53) (S)	×r13(6) (S)	6	5	0.2	S	r13(28) (S)	×r13(6) (S)	7	4	0.9	S
r13(23) (S)	×r13(6) (S)	7	6	0.1	S	r13(20) (S)	×r13(6) (S)	6	3	0	S
r13(17) (S)	×r13(6) (S)	7	0	0	S	r13(59) (S)	×r13(6) (S)	6	5	0.3	S
r13(7) (S)	×r13(6) (S)	5	5	1	S	r13(10) (S)	×r13(6) (S)	6	0	0	S
r13(27) (S)	×r13(6) (S)	6	0	0	S	r13(8) (F)	×r13(6) (S)	5	5	5.6	F
r13(16) (F)	×r13(6) (S)	6	6	7.2	F	r13(22) (F)	×r13(6) (S)	7	7	1.1	S
t10(9) (S)	×t10(11) (S)	6	3	0	S	t10(19) (S)	×t10(11) (S)	7	6	2.7	S
t10(21) (S)	×t10(11) (S)	9	0	0	S	t10(24) (S)	×t10(11) (S)	7	0	0	S
t10(23) (S)	×t10(11) (S)	7	7	1.1	S	t10(17) (S)	×t10(11) (S)	6	6	0.2	S
t10(10) (S)	×t10(11) (S)	7	7	0.7	S	t10(12) (S)	×t10(11) (S)	8	8	0	S
t10(14) (F)	×t10(11) (S)	6	4	2	S	t10(7) (F)	×t10(11) (S)	6	0	0	S
t10(13) (F)	×t10(11) (S)	7	7	12.7	F	t10(15) (F)	×t10(11) (S)	6	1	0.2	S
t11(50) (S)	×t11(45) (S)	5	5	0.4	S	t11(11) (S)	×t11(45) (S)	6	3	0	S
t11(21) (S)	×t11(45) (S)	5	5	1	S	t11(17) (S)	×t11(45) (S)	5	5	0	S
t11(7) (S)	×t11(45) (S)	6	0	0	S	t11(29) (S)	×t11(45) (S)	8	3	0.3	S
t11(37) (S)	×t11(45) (S)	5	3	0.2	S	t11(6) (F)	×t11(45) (S)	8	8	10.8	F
t11(36) (F)	×t11(45) (S)	6	6	12.5	F	.. ..	.. ..	..	..	..	..

TABLE 16

Inter-family crosses among self-sterile segregates.

v2(5)	×v3(1)	8	8	10.3	F	v2(15)	×t10(11)	5	5	7.2	F
v3(1)	×v5(61)	7	7	7.3	F	v5(61)	×v2(5)	8	0	0	S
r6(51)	×v3(1)	7	4	1.1	S	r6(51)	×v5(61)	6	6	13.3	F
r6(40)	×r13(6)	7	7	8.7	F	r6(59)	×v3(1)	7	0	0	S
r6(59)	×v5(61)	7	7	7.3	F	r7(7)	×v3(1)	6	6	14.2	F
r7(7)	×v5(61)	6	4	2.3	S	r7(7)	×r6(51)	5	5	11.2	F
r8(6)	×v3(1)	5	4	10.3	F	r8(6)	×v5(61)	5	1	0.2	S
r8(6)	×r6(51)	5	5	9.6	F	r12(20)	×v3(1)	6	6	14.2	F
r12(20)	×v5(61)	5	3	1.4	S	r13(53)	×v3(1)	7	7	5.6	F
r13(53)	×v5(61)	6	0	0	S	r13(6)	×v3(1)	6	6	8.6	F
r13(6)	×v5(61)	6	3	0.8	S	t5(21)	×v5(61)	6	1	0.2	S
t5(21)	×r6(51)	4	4	10.3	F	t8(75)	×r6(51)	6	6	3.3	F
t8(75)	×v5(61)	5	5	1.8	S	t8(75)	×v3(1)	7	6	4.3	F
t10(21)	×v3(1)	6	6	0.3	S	t10(21)	×v5(61)	6	6	9.7	F
t10(17)	×v3(1)	6	4	0	S	t11(3)	×v3(1)	6	6	8.2	F
t11(29)	×v3(1)	7	7	13.3	F	t11(29)	×v5(61)	5	5	1.6	S
t12(22)	×v3(1)	5	5	7	F	t12(22)	×v5(61)	7	0	0	S
t13(11)	×v3(1)	6	6	15.5	F	t13(11)	×v5(61)	6	0	0	S

TABLE 17

Crosses between self-sterile segregates and C. grandiflora.

31(1)×v3(1)	8	8	10.6	F	31(8)×v3(1)	7	0	0	S
31(3)×v5(61)	..	..	..	F	33(4)×t11(3)	6	6	14.7	F
33(7)×v3(1)	4	4	8	F	33(4)×v3(1)	7	0	0	S

INHERITANCE OF WILDNESS AND TAMENESS IN MICE[1]

WALKER MYRICK DAWSON
University of Illinois, Urbana, Illinois

Received September 9, 1931

TABLE OF CONTENTS

	Page
Introduction	296
Materials	297
Methods	298
Care of animals	298
Testing the mice in runway	298
Selection and mating of breeding stock	300
Results	301
Reliability of test	301
Parental strains	302
F_1 generation	305
F_2 generation	306
Backcrosses	306
F_3 and F_2 selections	307
Selection in the wild and tame stocks	307
Indications of linkage	310
Estimation of the number of genetic factors	310
Discussion	315
Summary and conclusions	315
Literature cited	316
Appendix	317

INTRODUCTION

Races and strains of domestic animals as well as individuals within a group often differ to a marked extent in temperament and reaction to a given situation. These differences are, in many cases, of considerable economic importance. For example, some breeds of chickens are much more excitable than others, draft horses generally have much quieter dispositions than race horses, dairy cows are as a rule more nervous and "temperamental" than beef cattle. Unfortunately little work has been done in studying the mode of inheritance of characters of this type.

[1] Paper No. 43 from the Division of Animal Genetics, Department of Animal Husbandry, University of Illinois, part of a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Husbandry in the Graduate School of the University of Illinois, 1931. The author wishes to express his appreciation to Doctor Elmer Roberts under whom the experiment was conducted and also to Professor C. M. Woodworth who assisted with the problem during Doctor Roberts' absence in China, 1929–30.

* The cost of the accompanying tables is paid by the Galton and Mendel Memorial Fund.

The purpose of this investigation was to determine whether genetic factors are involved in wildness and tameness in mice and if so the mode of their inheritance. In this experiment the length of time required by a mouse to travel a given distance under the conditions specified was taken as a measure indicating the degree of wildness or tameness. The use of these terms does not imply that the writer believes that the measure used in this study measures to the full extent wildness and tameness. These phenomena are very complex and the best that can be done is to measure some manifestations of them. This YERKES and COBURN did through observation and the writer has attempted to do by measuring the reaction of running under the conditions specified (p. 299 in which a significant difference existed between wild and domesticated strains).

It seems reasonable that wild mice have been selected for wildness by the process of natural selection and that tame mice have been selected for tameness by the process of domestication. Thus one would expect if wildness and tameness are inherited that there would be a tendency for the wild mice to be homozygous for the genes for wildness and the domesticated mice homozygous for the genes for tameness.

MATERIALS

There were two quite distinct types of mice in regard to their reactions available for a study of this kind: (1) the so-called wild mice found in barns and houses, and (2) the so-called tame mice sold by fanciers. The mice used to obtain stock for this experiment came from the following sources:

Wild mice

All of the wild mice came from a strain which had been reared for several years by Doctor ELMER ROBERTS in the Animal Genetics Laboratory of the UNIVERSITY OF ILLINOIS. These mice were all descendants of mice caught in the wild state at a considerable distance from the laboratory. They were easily excited and would run rapidly about their cage or hide in the paper when their pen was disturbed. When picked up they invariably struggled and tried to escape often biting the forceps or gloved hand with which they were held. If allowed to escape on the floor they would immediately dash for safety under the furniture or into a corner of the room. Sixteen pairs were used.

Tame mice

The tame mice were of three strains:

Albinos—Three males and twelve females were obtained from a fancier in New York City.

Pink eyed short eared browns—Two males and thirteen females were obtained from Professor W. H. GATES at LOUISIANA STATE UNIVERSITY.

Pink eyed browns—a pair of these was obtained from Dr. Elmer Roberts. They came from a strain that had been maintained in the laboratory for a number of years.

The mice in these three tame strains varied considerably in their reactions to being disturbed or caught but in general their motions were slower than those of the wild mice. They often did not attempt to hide and were much less likely to struggle or bite when caught. When placed on the floor they generally sniffed about curiously but did not run away as did the wild mice.

METHODS

Care of the animals

All of the mice were cared for in as nearly the same manner as possible throughout the experiment. The handling of the animals, in addition to testing them to determine their reaction, consisted in that necessary for weaning the young and for the transfer of mice in breeding operations. When matings were made between parents from strains which reacted very differently in the runway the male was always removed before the young were born or within one or two days after their birth. The breeding pens were inspected every day when young were expected. When the young were a month to six weeks old, they were weaned and records made of their color, sex, and length of ears where this character was involved. Young mice of the same sex were placed together in a separate pen until after completion of tests. Individuals from two or more litters were sometimes placed in the same cage, although mice that were expected to differ widely in their reaction in the runway were always placed in separate pens.

In handling the adult or partly grown mice during the above procedure and also during the tests, we caught the animals by the tail with a pair of placental forceps. If they were inspected or marked they were held in the gloved hand. Five-gallon tin cans with a few inches of shredded paper in the bottom were used as receptacles for the mice during the tests, when weaning the young and when making up matings.

Testing the mice in the runway

The method of testing consisted in placing the mouse at one end of a runway and allowing it to run to the other end. The time required was recorded by means of a stop watch.

The runway was 24 feet long, 9½ inches wide and 13 inches high. The sides and ends were of galvanized sheet iron, the floor of soft wood. One

foot from each end a black line was painted on the floor of the runway. The time required for the mouse to run from one line to the other, a distance of 22 feet, was recorded. A movable partition made of wallboard and bound with rubber was used to prevent the mouse from running back during the test and to aid in starting the test and capturing the mouse afterward.

The following procedure was carried out in testing the mice in this device. On the date that the mice in a certain pen were to be tested they were carried to the runway a short distance away and tested one at a time. The mouse to be tested was confined by the movable partition in a space about one foot from the end of the runway until everything was ready when the partition was raised and the stop watch started as soon as the mouse crossed the black line. The mouse was followed by the experimenter with the partition which was placed in position to prevent the animal from running back if it showed any signs of doing so. Nothing was done to frighten the mouse other than the procedure described. This was usually sufficient to cause even the tame mice to run or walk toward the other end of the runway. If the mouse ran swiftly, it was impossible to keep up with it with the partition; but if more slowly the partition was moved along and kept about twelve to fifteen inches behind the mouse. If the mouse stopped and showed no inclination to go forward the partition was slowly advanced until it touched the mouse. In all but three or four cases this was sufficient to start the mouse again. The few individuals where this was not the case were shoved a little and thus started. When the mouse crossed the line at the far end of the runway the watch was stopped and the partition taken out of the runway allowing the mouse to run back to the starting point or in case it did not do so voluntarily it was urged by means of the partition. This prevented the mice from associating the far end of the runway with being caught. Since each individual was tested three times this point was of considerable importance.

After the mouse had been cornered at the starting point by means of the partition, it was caught and the number in its ear read. The length of time required to run the 22 feet was then recorded together with any notes deemed necessary. When all the mice in the pen had thus been tested, they were taken back into the room where they were permanently kept and weighed individually to the nearest quarter of a gram.

Each mouse in the experiment was tested by the above method three times. These trials were conducted at weekly intervals after the mouse reached 75 days of age. In order to facilitate the testing and caring for the mice, a variation of one day in either direction was permitted. Thus

the first trial for a given mouse might occur on the 74th, 75th or 76th day. A few trials had to be made on different dates.

The trials were in nearly all cases made in the evening or at night when there was very little outside disturbance to distract the mice. The lighting was kept as far as possible the same throughout the experiment.

Selection and mating of breeding stock

Mice of the various stocks described on page 297 were mated and the young raised under the conditions in the laboratory to produce the parental individuals used in the experiment. These animals have been designated as first generation wild and first generation tame. As an aid in selecting breeders to use in crosses a preliminary study was made of the spread and nature of the population curves of the wild and tame strains. The average of the three trials in the runway was used as the basis of selection throughout the experiment. Other factors, however, were considered such as the health of the individual, nature of the matings, spread of the items making up the average or anything which indicated that the average might not truly represent the genetic constitution of the individual.

Breeders to be used in producing F_1's were chosen from the fastest and slowest parts of the wild and tame distributions respectively (figures 1 and 2). In the wild strain breeders were selected that ran the distance in less than six seconds while in the tame strain they were selected from those that had a record of twenty seconds or slower. It was believed that by this procedure there would be a tendency to select individuals more nearly homozygous for the factors controlling the reaction than there would be if individuals were selected at random in case the population of the strain contained heterozygous individuals. If, on the other hand, the strain were homozygous, no harm would be done by the selection.

Reciprocal matings were used in producing the F_1's. After a considerable number of individuals were obtained a study was made of their distribution with regard to their reaction in the runway in order to determine the proportion of individuals at various parts of the distribution. F_2's were produced from a group of F_1's selected from the whole F_1 population in such a manner that the proportion of mice having a certain average test in the runway would conform as nearly as possible to that in the entire population. Backcrosses were made between F_1's which varied in the degree of their reaction and both parental strains. A number of female individuals from $F_1 \times$ tame were mated back to tame males, often to their sires. This formed in reality a progeny test of the females. Matings were

made between individuals from various parts of the F_2 population, and the progeny tested in some cases for several generations.

Inbreeding was avoided as much as possible in the production of the first generation wild, first generation tame, F_1's and F_2's. The descendants of the various tame strains were kept separate in crosses; for example, F_1's from wild×albino were always mated to F_1's from wild×albino in producing the F_2's.

RESULTS

The results given were obtained over a period of three and a half years from the fall of 1926 to the spring of 1930. Two thousand three hundred seventy-six mice were born in the experiment and of these one thousand five hundred seventy-five were raised to maturity and tested in the runway. Nearly all of these entered into the results presented, although some on which complete records could not be obtained or which were derived from special crosses, as $F_2 \times F_2$, have been omitted.

Very little if any difference was found between the tame strains. In most cases they have been considered together in the results, although in a number of instances only the albino strain has been used. More than two-thirds of the tame population in the first generation were albinos. They proved much more vigorous and prolific than either of the other tame strains and for this reason were used to a greater extent.

Reliability of test

The reliability of the runway test appears to be satisfactory, at least when the results from all the mice are judged by (1) correlating the different trials with each other and (2) obtaining the coefficient of reliability by use of the Spearman Brown formula.[2] SHEN's (1924) formula was used to compute the probable errors of the reliability coefficients[3] (tables 1 and 1a).

The distribution of the mice in scatter diagrams for the correlation between the trials for all the mice indicates that the correlation is rectilinear or nearly so. Correlation ratios were worked out for the wild males first generation for the three trials in the runway.[4] Correlations of all except the third trial on the first satisfied the Blakeman test (REITZ 1927), and this exception exceeded the limit set by BLAKEMAN by only a small amount.

[2] Spearman Brown formula: $R = ar/1+(a-1)r$ in which R = the coefficient of reliability when the original test is used (a) times; r = the average of the correlation coefficients of the (a) trials. (KELLEY 1923, ODELL 1930.)

[3] P. $E._R = 0.6745\ a(1-r)^2/\sqrt{N}[1-(a-1)_r]^2$ (SHEN 1924).

[4] $\eta_{12} = 0.738$ $\eta_{13} = 0.618$ $\eta_{23} = 0.860$
$\eta_{21} = 0.848$ $\eta_{31} = 0.712$ $\eta_{32} = 0.838$

TABLE 1

Reliability of the runway test for various groups of mice.

GROUP	NUMBER OF MICE	COEFFICIENT OF RELIABILITY*
All mice used	1232	.0.921±0.003
All ♂♂	614	0.927±0.004
All ♀♀	618	0.923±0.004
Wild and Albino first generation ♂♂	82	0.957±0.007
Wild and Albino first generation ♀♀	88	0.967±0.007
Wild first generation ♂♂	43	0.827±0.038
Albino first generation ♂♂	39	0.709±0.071
Wild first generation ♀♀	47	0.866±0.028
Albino first generation ♀♀	41	0.873±0.028

* See footnotes 2 and 3 for the formulas used.

TABLE 1a

Correlation coefficients between the three trials in the runway for various groups.

GROUP	TRIALS CORRELATED			
	NUMBER OF INDIVIDUALS	FIRST WITH SECOND TRIAL	FIRST WITH THIRD TRIAL	SECOND WITH THIRD TRIAL
All mice used	1232	0.788±0.007	0.759±0.008	0.841±0.006
All ♂♂	614	0.810±0.009	0.788±0.010	0.827±0.009
All ♀♀	618	0.764±0.011	0.750±0.012	0.885±0.006
Wild and Albino first generation ♂♂	82	0.878±0.017	0.851±0.021	0.915±0.012
Wild and Albino first generation ♀♀	88	0.909±0.012	0.883±0.016	0.929±0.010
Wild first generation ♂♂	43	0.687±0.054	0.390±0.087	0.790±0.039
Albino first generation ♂♂	39	0.403±0.090	0.267±0.100	0.676±0.059
Wild first generation ♀♀	47	0.676±0.053	0.658±0.056	0.716±0.048
Albino first generation ♀♀	41	0.696±0.054	0.599±0.068	0.791±0.039

Parental strains

The results of testing the first generation wild and first generation tame in the runway are given in tables 2 and 3 and figures 1 and 2. (Animals of the first generation are unselected, see page 300.) These are for the average of three trials. The tables and figures show that there is a marked difference between the wild and tame strains. A comparison of the means shows that the difference is more than 24 times its probable error. There is no overlapping in the males and very little in the females. The variability

of the wild is very much less than that of the tame. A study of the difference between the sexes, given in table 3, shows that in the wild strain the females are significantly faster than the males, while in the tame strains the difference is not significant except in the first trial for the albinos. In

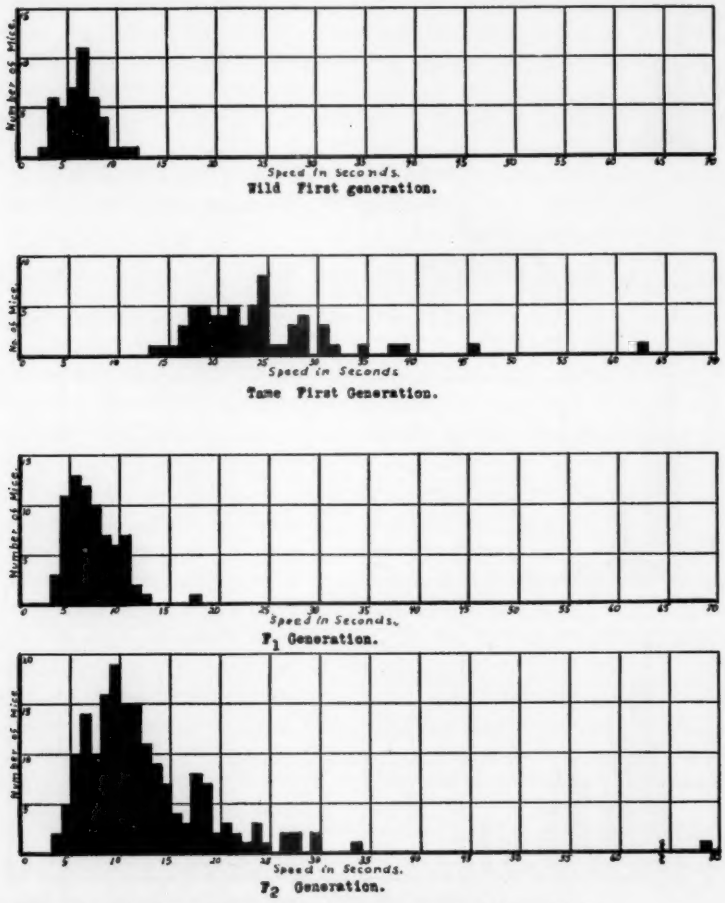


FIGURE 1.—Distribution of males in runway test. Average of three trials.

this case the males are significantly faster than the females. The smaller number of animals in the pink eyed short eared brown strain probably accounts for the difference not being significant in that case.

The distributions of the wild and albino tame strains for the first generation in the first, second and third trials in the runway are given in

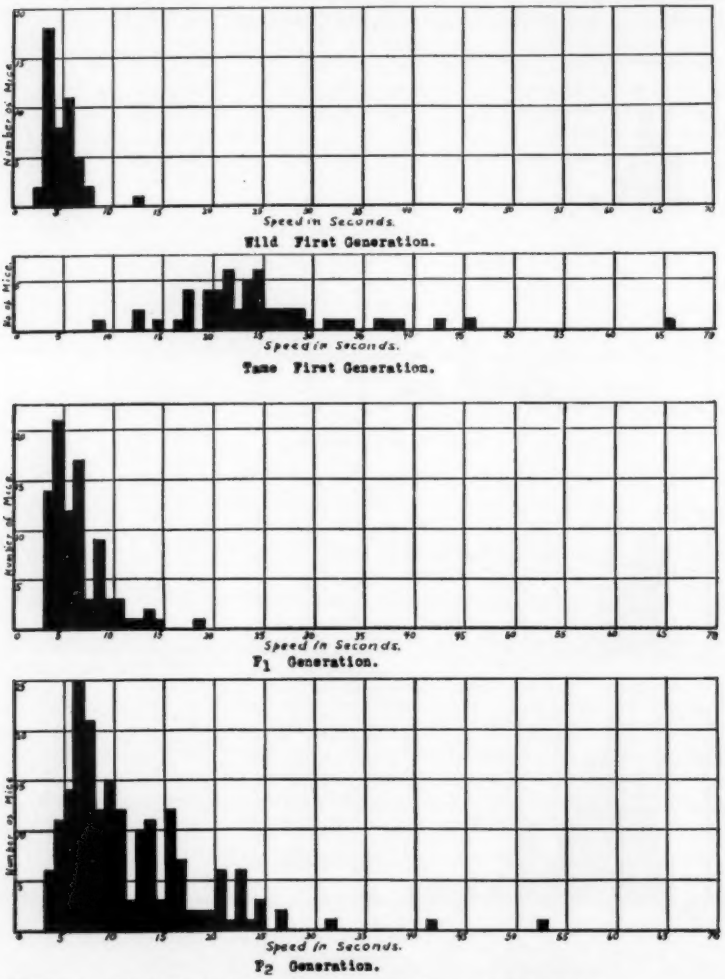


FIGURE 2.—Distribution of females in runway test. Average of three trials.

table 5. It is interesting to note that the mean speed in each case is lowest in the first trial and increased in the second and third trials. In the tame

strains the difference between the first trial and the second and third trials is very marked and is proportionally about twice as large as in the wild strain.

The correlations between the trials as shown by the correlation coefficients in table 1a are fairly high. When the wild and tame stocks are treated as one population the correlation between the trials is greater than when the wild and tame stocks are considered separately, that is as two populations. This difference can be explained upon the basis of the existence of unlike sub populations in the combined table which in this case are the wild and tame strains.

Weight and age were found to have little effect on reaction when studied with the wild and albino strains in the runway.

F_1 generation

The distribution of F_1's produced from crossing selected individuals of the wild and tame strains (described under Methods, page 300) is given in tables 2 and 3 and figures 1 and 2. The reaction of the wild mice was found to be almost completely dominant over the reaction of the tame mice. The mean for the F_1's showed them to be very little slower than the wild. While the variability of the F_1's was slightly greater than that of the wild, the population was quite uniform. The difference between the sexes (table 3) was not significant. However, it is interesting to note that the mean for the females is slightly less than that for the males.

No difference that could be considered significant was found between F_1's from reciprocal crosses. A comparison of the means is given in table 6. The fact that no difference existed between the young from the two types of matings shows that the association with the mother had had practically no effect on the speed of the offspring.[5] At least the offspring of of tame mothers mated to wild males tested much faster than their mothers. Also, as will be seen later, fast F_1 females produced some slow F_2 offspring, thus proving that the results obtained can not be accounted for by the influence exerted by the association of the young with parents of different degrees of reaction in the runway.

F_1 matings were studied individually to see whether the degree of reaction shown by the F_1 parents had a noticeable effect on the distribution of the F_2 offspring. There seemed to be a slight tendency for the slower F_1's to produce slower offspring, but on the whole the results were quite uniform. However, the faster F_1 parents were found to have produced

[5] The father was removed from the pen before he could influence the young.

proportionately slightly more of the tested F_2 population than were produced by the slower F_1 parents.

F_2 generation

All of the F_2 population which reached 75 days of age was tested in the runway. Segregation of the general behavior of the mice was quite apparent from observation as well as from results of the test. Animals were often found in the same litter which reacted very differently when picked up and examined in the hand.

Table 2 and figures 1 and 2 show the F_2 distribution for the average of three trials in the runway. As can be seen there is a very wide spread, ranging from almost as fast as the fastest grandparents to slower than the slowest grandparent. The distribution was considerably skewed toward the left as was the case in the F_1. The mean, however, occupied a position which was intermediate between that of the F_1 and that of the tame although the average F_2 was not as slow as the parental average. A significant increase in variability was obtained in the F_2 over that in the F_1, showing that considerable segregation had taken place (table 4). There was only one prominent mode in the F_2 distribution. The sexes did not show a significant difference, at least when the difference between the means was compared to the probable error (table 3). The data in this respect were similar to those of the F_1's. Recombinations of other characters with wildness or tameness are discussed under indications of linkage (page 310).

Backcrosses

The results of testing offspring from backcross matings to the wild and tame parental strains are given in table 7. It will be noted that there is a great difference in the distribution of the young from the two types of matings. These distributions were in accord with expectation in that the population of the backcross to the dominant wild lay entirely within the range occupied by the wild and F_1's and was very uniform. Also the population of the backcross to the recessive tame extended from the F_1 range to the tame range and was very variable.

However, it will be noticed that the distribution of the backcross to the wild is extremely skewed toward the wild and that in the distribution of the backcross to the tame none of the individuals are as fast as the fastest F_1's while the slowest are slower than the slowest parent used. Heterozygosity for the factors for a slow reaction in the tame parent together with the difference between the tame individuals used in the original cross with

the wild and those used in the backcross would account for these discrepancies. Tame mice from the first generation were used in the original cross, while individuals of the second and third generation were largely used for the backcross.

In addition to the above, results from progeny tests on females in backcrosses to tame males were also obtained. From matings of this type one would expect the offspring of different females to vary in degrees of wildness from that of the mother to that of the father. In most cases the speed of the fastest offspring approximated the speed of the mother (table 8). However, the four slowest females did produce some young considerably faster than themselves and three of the seven males used produced some offspring slower than themselves. These unexpected deviations may be accounted for by a number of suppositions such as inaccuracy of the test, presence of complementary factors or the occurrence of some dominant genes for the reaction in the tame males. (These must be modifying genes.) The latter explanation appears to be the best in view of the results obtained by selection within the tame stock.

F_2 and F_3 selections

The results of selecting and mating F_2's and F_3's having different reactions in the runway in an attempt to establish strains that would breed true are given in table 9. It will be noted that the offspring from the first mating given are all within the limits of the original wild stock. Also a mating of two of these offspring, the first mating given for the F_3's, produced offspring all of which were also within the limits of the original wild strain. The offspring from all the other matings showed considerable variation. In general, though, the slower the parents were the slower the offspring. None of the offspring of the slowest parents were within the range of the wild. The small number of offspring from many of these matings made it impossible to consider the results from them conclusive.

Selection in the wild and tame stocks

Table 10 and figures 3 and 4 give the means for four generations of selection on the wild and tame stocks. In the wild strain the fastest individuals were selected, while in the tame strain the slowest were selected for breeders. It is clearly evident that selection had a marked effect on the tame strains. This progress towards a slower reaction by means of selection shows clearly that the original tame stocks were probably heterozygous for modifying factors for the reaction. (The tame mice must of necessity be homozygous for the recessive allelomorph of the principal gene or

genes controlling wildness; otherwise they would be wild like the F_1's.) This has also been shown in the F_2 and backcrosses to the tame where progeny slower than the tame parent were produced. On the other hand,

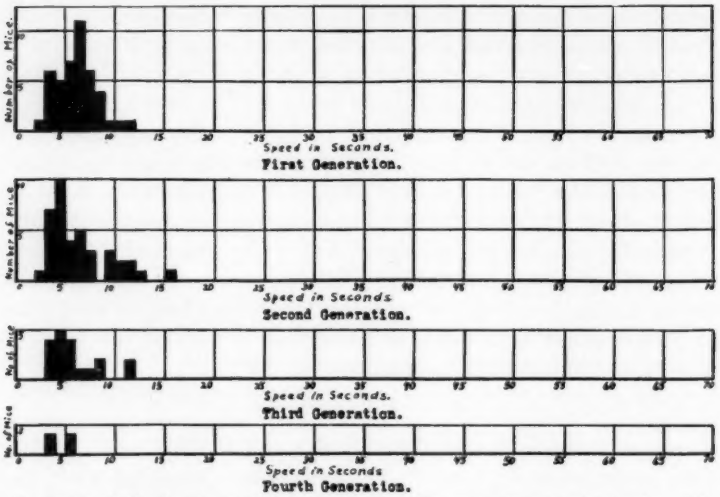


FIGURE 3a.—Distribution of wild males in succeeding generations of selection for wildness. Average of three trials.

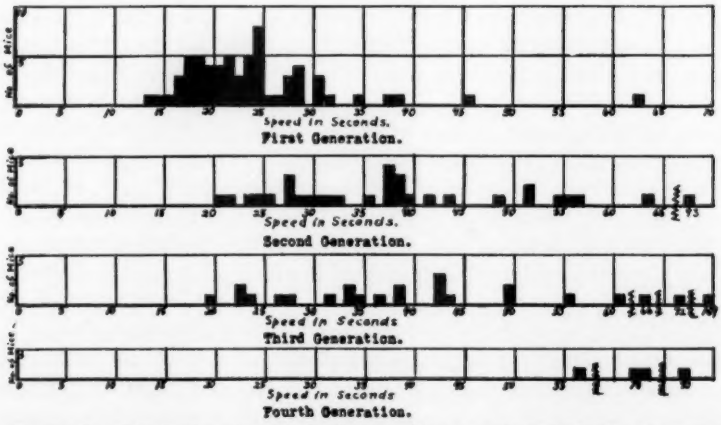


FIGURE 3b.—Distribution of tame males in succeeding generations of selection for tameness. Average of three trials.

selection seems to have had very little effect on the wild strain. Since wildness proved to be almost completely dominant over tameness, it would take a long time to produce any effect by selection even with heterozygous individuals present in the population. Selection of the fastest indi-

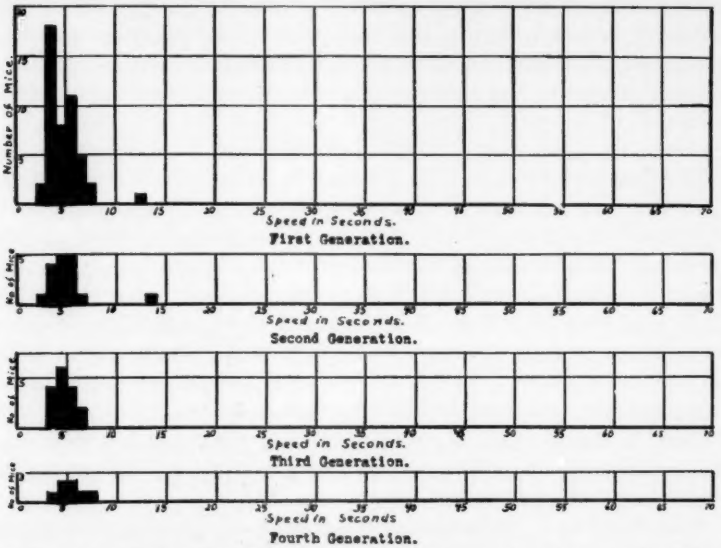


FIGURE 4a.—Distribution of wild females in succeeding generations of selection for wildness. Average of three trials.

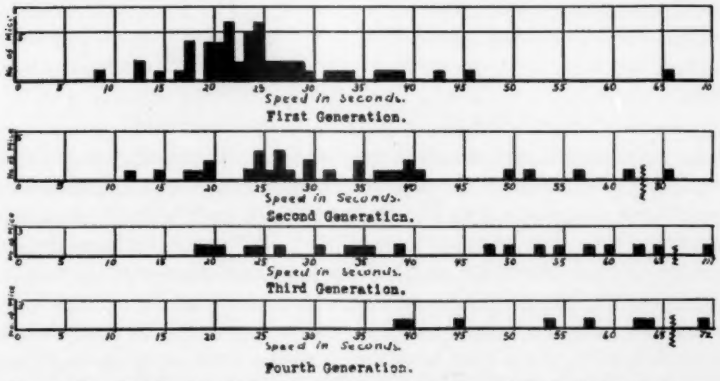


FIGURE 4b.—Distribution of tame females in succeeding generations of selection for tameness. Average of three trials.

viduals for breeders and the elimination of any recessives which might appear could be expected to produce a more homozygous population for the genes which cause the mice to run fast by the gradual reduction of the number of heterozygous individuals present. It might not be possible to demonstrate this effect except by breeding tests unless large numbers were used. The fact that the wild had been subjected for many generations to natural selection would lead one to expect them to be homozygous for all or nearly all the factors which would cause the mice to run quickly in the runway.

Indications of linkage

The data from the F_2 and backcrosses to the tame have been analyzed for indications of linkage by comparing the means of the groups showing the recessives albinism, brown, pink eye, non-agouti, and short ear with the mice having the normal allelomorph of the character in question. The results from the F_2 are given in table 11, those from the backcross in table 12. It can be seen that if any linkage existed it was slight. The differences which appeared to be significant in the F_2 did not show the same relationship in the backcross. Since there were no pink eyed short eared browns in the backcross and since the F_2 population was not separated into strains, a direct comparison in this way is subject to error. Further work along this line might be profitable when larger numbers are involved and the experiment is planned to test linkage.

From table 11 it appeared that the number of recessive genes present might have an effect on the reaction of the individual. When groups of F_2's homozygous for one, two or three of the recessive genes non-agouti, brown and pink eye were studied, the individuals with the most recessives seemed to be somewhat slower than individuals having the dominant allelomorphs. More data, however, would be necessary for definite conclusions.

Estimation of the number of genetic factors

An attempt has been made to estimate the number of genetic factors responsible for the results obtained in the runway. The fact that parental types were so quickly obtained in the F_2 with such a small population would indicate that the greater part of the difference between the wild and the tame strains is due to relatively few genes. However, the response to selection in the tame, the extreme skewness of the distribution in the F_2 and the lack of distinct modes in the backcross to the tame indicate that while a few genes may largely control the response of the individual yet a rather large number have some influence.

In attempting to determine the number of genes which largely control

the response of the animal, the population of the second generation of wild and tame strains, F_1's and the backcrosses to the wild and tame have been given values proportionate to their occurrence as genotypic types in the F_2 when Mendelian ratios for one, two and three gene differences are taken. The second generation of the wild and tame strains was used because it would show the effect that selection could be expected to play in the original cross. The combination of the wild, F_1 and tame populations in the proportion $\frac{1}{4}+\frac{1}{2}+\frac{1}{4}$ should give the expected F_2 distribution for a one factor difference. If the parent types were pure this could be expected to approximate closely the observed. For a two factor difference the backcrosses to the tame and wild can be used to supply the distribution of classes not given by the parental or F_1 strains. Because dominance is so nearly complete it can be assumed that classes such as *aaBB* and *aaBb* are practically identical. When more than two or three factors are assumed to have any considerable effect, these classes, accounted for by the assumption of dominance, become more important and where dominance is not complete probably sufficient error is introduced to make it impractical to use the method.

The distributions expected in the F_2 are shown in figures 5, 6 and 7, when one, two and three gene differences are involved. The method of adding together the components which make up the F_2 population as described above was used. The actual F_2 distribution is also given and a comparison of the means of the two curves made.

The probability that the difference between the means was due to chance was greatest when a difference of two genes was used as the basis of the expected F_2 distribution. When the χ^2 test for goodness of fit was used the probability that the difference between the curves was due to chance was very low in all cases. The best fits were secured when a difference of two genes was used in the males and when a difference of three genes was used in the females. It should be noted, however, that the general shape of the expected curve seems to correspond somewhat better when a difference of one gene was used than when two or three genes were used in the case of the males. A satisfactory explanation for this has not been discovered.

In each of the three cases the expected F_2 distribution has more individuals at the extremes and less in the region from about eight to fourteen seconds than the actual F_2 population. The region from eight to fourteen seconds in the expected F_2 distribution is made up almost entirely of F_1's, the slowest individuals from the backcross to the wild and the fastest individuals from the backcross to the tame.

An examination of the backcross distributions shows in both cases that there are fewer individuals within the range eight to fourteen seconds than would be expected if the backcross had produced all classes in equal proportion. Theoretically, the fastest individuals from the backcross to the tame should coincide with the fastest F_1's while the slowest individuals from the backcross to the wild should coincide with the slowest F_1's if the

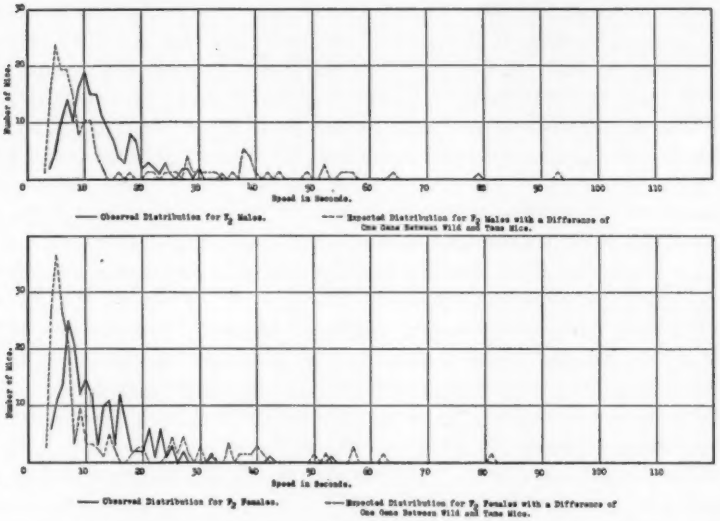

Number of Mice.
Speed in Seconds.
Observed Distribution for F2 Males.
Expected Distribution for F2 Males with a Difference of One Gene Between Wild and Tame Mice.
Number of Mice.
Speed in Seconds.
Observed Distribution for F2 Females.
Expected Distribution for F2 Females with a Difference of One Gene Between Wild and Tame Mice.

FIGURE 5.—Comparison of the observed F_2 distributions for males and females with those expected for a difference of one gene between the wild and tame mice.

In the expected F_2 distribution the wild second generation = 1/4, the F_1 = 1/2, the tame second generation = 1/4.

The means for the distributions were:

	Males	*Females*
Expected	15.985±0.576	13.413±0.701
Observed	12.949±0.387	11.853±0.331
Difference	3.036±0.694	1.560±0.775
Diff./P.E.	4.4	2.6

parental stocks used in the original cross and in the backcrosses were of the same genetic constitution. The fact that the overlap between the wild and tame backcrosses covers a somewhat less extended range than the distribution of the F_1's (tables 2 and 7) suggests that the parental stocks used in the original cross and those used in the backcrosses did differ

genetically. Under these conditions a divergence of the expected and actual F_2 distributions would be expected.

If heterozygosity is introduced in one of the parents, as for example a cross between $AABB \times Aabb$, the F_1's will consist of two types which if dominance is present will be very similar to the dominant parent and to each other. Random matings between these two types, however, would give in the F_2, instead of a 9:3:3:1 ratio, a ratio of 45:15:3:1. If the population were not very large this would appear like a 3:1 ratio with perhaps one or two exceptional individuals. With three pairs involved and one pair heterozygous in the recessive parent, as for example in the mating $AABBCC \times Aabbcc$ the F_1 would still have two types and the F_2 would give a ratio of 135:45:45:15:9:3:3:1 assuming dominance. If the population were small the bulk of this distribution could be expected to re-

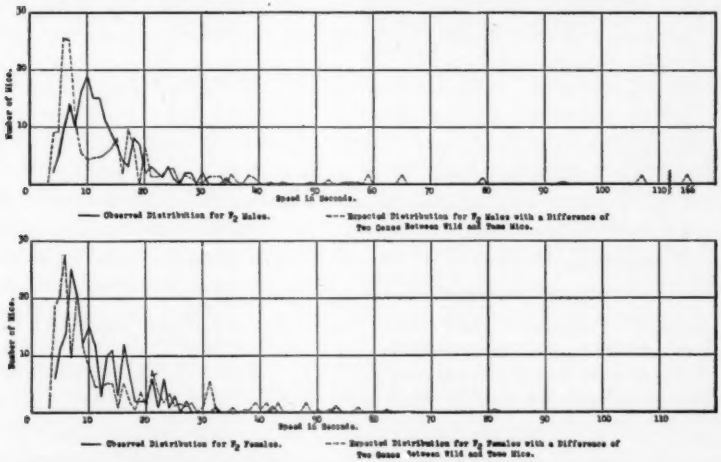

Number of Mice.
Speed in Seconds.
Observed Distribution for F2 Males.
Expected Distribution for F2 Males with a Difference of Two Genes Between Wild and Tame Mice.
Number of Mice.
Speed in Seconds.
Observed Distribution for F2 Females.
Expected Distribution for F2 Females with a Difference of Two Genes between Wild and Tame Mice.

FIGURE 6.—Comparison of the observed F_2 distributions for males and females with those expected for a difference of two genes between the wild and tame mice.

In the expected F_2 distribution the wild second generation = 1/16, the $F_1 \times$ wild (F_1 = 1/4 + wild = 1/4) = 1/4, the F_1 = 1/4, the $F_1 \times$ tame (F_1 = 1/4 + tame = 1/4) = 3/8, the tame second generation = 1/16.

The means for the distributions were:

	Males	*Females*
Expected	16.348±1.052	13.007±0.542
Observed	12.949±0.387	11.853±0.331
Difference	3.399±1.122	1.154±0.636
Diff./P.E.	3.0	1.8

semble a 9:3:3:1 ratio. From these examples it can be seen that the introduction of heterozygosity in the parents lowers the gametic differences and therefore will cause the F_2 population in similar cases to appear to have a lower number of genes involved than is actually the case.

The above discussion is based upon the assumption that there is equal viability for the gametes from the different F_1 types. In case there is a difference, as, for example, if the gametes from *AABb* were more viable than those for *AaBb*, then the resulting distribution would approach that

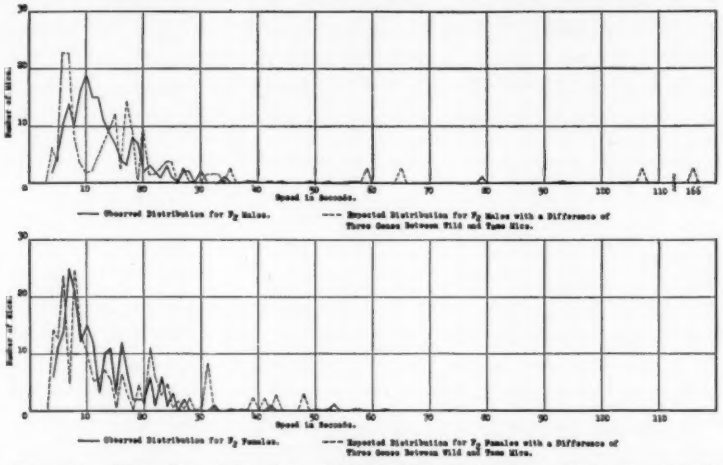


FIGURE 7.—Comparison of the observed F_2 distributions for males and females with those expected for a difference of three genes between the wild and tame mice.

In the expected F_2 distribution the wild second generation = 1/64, the $F_1 \times$ wild (F_1 = 1/8 + wild = 1/8) = 18/64, the F_1 = 1/8, the $F_1 \times$ tame (F_1 = 1/8 + tame = 1/8) = 36/64, the tame second generation = 1/64.

The means for the distributions were:

	Males	*Females*
Expected	18.760±1.208	14.018±0.510
Observed	12.949±0.387	11.853±0.331
Difference	5.811±1.268	2.165±0.608
Diff./P.E.	4.6	3.6

of a single factor difference more nearly than if the viability were equal. On the other hand, if the gametes from *AaBb* were the more viable the distribution would approximate more closely the distribution for a two factor difference.

The effect of unequal numbers of the different types in the F_1 would be similar to that for unequal viability of the gametes.

Applying the above reasoning to the results from the runway tests the following appear probable:

1. That there are two or three genes involved that have considerable influence on the reaction.[6]
2. That practically all the wild parents were homozygous for the dominant genes.
3. That most of the tame parents were homozygous for at least two recessive genes and heterozygous for the other.
4. That the unequal fertility noted in the F_1's coupled with the heterozygosity of one or more pairs of genes in the tame parent has caused the distribution of the F_2 to be skewed somewhat more toward the left than it would be otherwise.
5. That the variation from the distribution expected with a difference of two genes is compatible with the results of selection in the tame strains.

DISCUSSION

A number of other experimenters have attempted to study the inheritance of wildness and tameness or characters of a somewhat similar nature. The results obtained in the present investigation, in spite of differences in methods and materials, agree at least in certain respects with those of YERKES (1913) and COBURN (1922), both of whom found wildness and tameness to be inherited and that wildness was largely dominant over tameness, and also those of VICARI (1929) who, in studying the inheritance of reaction time and degrees of learning in mice, found that where there was a wide difference between short and long reaction time some form of dominance of the short reaction time was evident. Others who have studied the inheritance of characters of this type are BAGG (1920), TOLMAN (1924) and SADOVNIKOVA-KOLTZOVA (1926).Their results all give some evidence that inheritance affects the characters studied but, at least from the data presented, appear to give very little information on the mode of inheritance.

SUMMARY AND CONCLUSIONS

Whether the characters wildness and tameness in mice as measured by the results of testing in the runway are inherited was studied by making

[6] CASTLE (1921) has proposed a formula ($n = D^2/8[\sigma_2^2 - \sigma_1^2]$) for estimating the number of genes concerned in cases of blending inheritance. While the application of this formula to the present material may be questioned, yet it is interesting to note that the estimated number of genes arrived at by this method is 2.6 for the males and 2.7 for the females, when D in the above formula equals the difference between the mean of the wild second generation and the mean of the tame second generation (see third paragraph, page 311).

appropriate crosses. One strain of wild mice and three strains of tame mice were used in the experiment. That factors for wildness and tameness are inherited is shown by the following:

1. A distinct difference was found between the wild and tame stocks. This difference was increased by selecting the parents through four generations.
2. The F_1 offspring were very nearly as fast as the wild parent.
3. The results of mating F_1's back to the wild stock were very different from those obtained by mating the F_1's to the tame stock.
4. Segregation similar to that of other characters which are inherited was obtained when the F_1's were mated *inter se* and to the tame stock.
5. The association of the young with the mother had no effect on their reaction when tested in the runway.

As to the mode of inheritance of the genes controlling the characters wildness and tameness, the most important facts disclosed are:

1. Only a few genes appear to influence the reaction to any great extent.
2. The genes which are responsible for wildness seem to be almost completely dominant to those which are responsible for tameness.
3. There is very little evidence from the results that any of these genes are linked with sex.
4. There is very little evidence that there is any linkage between these genes and those for albinism, pink eye, agouti, brown, or short ear.
5. Continued selection showed that probably a number of modifying genes are present.

LITERATURE CITED

BAGG, H. J., 1920 Individual differences and family resemblances in animal behavior. Arch. Psychol. New York **26**: 58.

CASTLE, W. E., 1921 An improved method of estimating the number of genetic factors concerned in cases of blending inheritance. Science **54**: 223.

COBURN, C. A., 1922 Heredity of wildness and savageness in mice. Behav. Monog. **4**: 1–71.

KELLEY, T. L., 1923 Statistical method. New York: The Macmillan Co.

ODELL, C. W., 1930 Educational measurement in high school. New York: The Century Co.

RIETZ, H. L., 1927 Mathematical statistics. Chicago: Open Court Publishing Co.

SADOVNIKOVA-KOLTZOVA, M. P., 1926 Genetic analysis of temperament of rats. J. Exp. Zool. **17**: 342–359.

SHEN, E., 1924 The standard error of certain estimated coefficients of correlation. J. Educ. Psychol. **15**: 462–465.

TOLMAN, E. C., 1924 The inheritance of maze ability in rats. J. Comp. Psychol. **4**: 1–18.

VICARI, E. M., 1929 Mode of inheritance of reaction time and degrees of learning in mice. J. Exp. Zool. **54**: 31–88.

YERKES, R. M., 1913 The heredity of savageness and wildness in rats. J. Anim. Behav. **8**: 286–296.

TABLE 2

Distribution of the first generation wild, first generation tame, F_1's and F_2's in runway test. Average of three trials.

SPEED IN SECONDS	MALES				FEMALES			
	WILD FIRST GENERATION	TAME FIRST GENERATION	F_1's	F_2's	WILD FIRST GENERATION	TAME FIRST GENERATION	F_1's	F_2's
3	1	..	..	..	2	..	..	..
4	6	..	6	2	18	..	14	6
5	5	..	11	5	8	..	21	11
6	7	..	13	10	11	..	12	14
7	11	..	12	14	5	..	17	25
8	6	..	10	10	2	..	3	21
9	4	..	7	16	..	1	9	12
10	1	..	6	19	..	..	3	15
11	1	..	7	15	..	..	3	12
12	1	..	2	15	..	..	1	3
13	..	..	1	11	1	2	1	10
14	..	1	..	9	..	..	2	11
15	..	1	..	7	..	1	1	3
16	..	1	..	4	..	..	..	12
17	..	3	..	3	..	1	..	7
18	..	5	1	8	..	4	..	2
19	..	5	..	7	..	..	1	2
20	..	4	..	2	..	4	..	2
21	..	4	..	3	..	4	..	6
22	..	5	..	2	..	6	..	1
23	..	3	..	1	.	2	..	6
24	..	5	..	3	..	5	..	1
25	..	8	..	1	..	6	..	3
26	..	1	..	..	..	2	..	..
27	..	1	..	2	..	2	..	2
28	..	3	..	2	..	2	..	..
29	..	4	..	..	..	2	..	..
30	..	..	..	2	..	1	..	..
31	..	3	..	..	..	..	..	..
32	..	1	..	..	..	1	..	1
33	..	..	..	..	..	1	..	..
34	..	..	..	1	..	1	..	..
35	..	1	..	..	..	..	..	..
36	..	..	..	..	..	..	..	..
37	..	..	..	..	..	1	..	..
38	..	1	..	..	..	1	..	..
39	..	1	..	..	..	1	..	..
40	..	..	..	..	..	..	..	..
41	..	..	..	..	..	..	..	..
42	..	..	..	..	..	..	..	1
43	..	..	..	..	..	1	..	..
46	..	1	..	..	..	1	..	..
53	..	..	..	..	..	..	..	1
63	..	1	..	..	..	..	..	..
66	..	..	..	..	..	1	..	..
79	..	..	..	1	..	..	..	..
Total	43	63	76	175	47	54	88	190
Mean	6.698 ±0.202	24.492 ±0.654	7.579 ±0.198	12.949 ±0.387	5.277 ±0.167	25.352 ±0.825	6.898 ±0.203	11.853 ±0.331

TABLE 3

Comparison of wildness of males and females in the wild first generation, tame first generation, F_1's and F_2's in the runway test. Average of three trials.

STRAIN	TRIAL	NO. OF IND.	MEAN FOR MALES	NO. OF IND.	MEAN FOR FEMALES	DIFFERENCE	D/P.E.
Wild	1	43	8.116±0.298	47	6.000±0.193	2.116±0.355	6.0
Wild	2	43	6.302±0.221	47	4.936±0.207	1.366±0.303	4.5
Wild	3	43	5.651±0.189	47	4.660±0.174	0.991±0.257	3.9
Wild	Average	43	6.698±0.202	47	5.277±0.167	1.421±0.263	5.4
Albino	1	39	31.436±0.872	41	36.732±0.127	5.296±0.881	6.0
Albino	2	39	19.385±0.506	41	18.976±0.717	0.409±0.877	0.5
Albino	3	39	16.154±0.502	41	15.829±0.565	0.325±0.756	0.4
Albino	Average	39	22.359±0.486	41	23.927±0.760	1.568±0.902	1.7
Pink-eyed short eared brown	1	20	38.650±2.209	11	45.727±5.148	7.077±5.602	1.3
	Average 3 Trials	20	27.900±1.525	11	31.182±2.566	3.282±2.985	1.1
Pink eyed brown	Average 3 Trials	4	28.250±3.492	2	22.500±0.239	5.750±3.500	1.6
All Tame	Average 3 Trials	63	24.492±0.654	54	25.352±0.825	0.860±1.054	0.8
F_1's	Average 3 Trials	76	7.579±0.198	88	6.898±0.203	0.680±0.284	2.4
F_2's	Average 3 Trials	175	12.949±0.387	190	11.853±0.331	1.096±0.510	2.1

TABLE 4

Comparisons of the means and standard deviations for various groups, average of three trials.

GROUP	NUMBER OF INDIVIDUALS	MEAN SPEED	GROUP	NUMBER OF INDIVIDUALS	MEAN SPEED	DIFFERENCE	D/P.E.
Wild first generation ♂♂	43	6.70±0.20	Tame first generation ♂♂	63	24.49±0.65	17.79±0.68	26.2
Wild first generation ♀♀	47	5.28±0.17	Tame first generation ♀♀	54	25.35±0.82	20.07±0.84	24.0
Wild first generation ♂♂	43	6.70±0.20	F_1 ♂♂	76	7.58±0.20	.88±0.28	3.1
Wild first generation ♀♀	47	5.28±0.17	F_1 ♀♀	88	6.90±0.20	1.62±0.26	6.2
Tame first generation ♂♂	63	24.49±0.65	F_1 ♂♂	76	7.58±0.20	16.91±0.68	24.9
Tame first generation ♀♀	54	25.35±0.82	F_1 ♀♀	88	6.90±0.20	18.45±0.84	22.0
F_1 ♂♂	76	7.58±0.20	F_2 ♂♂	175	12.95±0.39	5.37±0.44	12.2
F_1 ♀♀	88	6.90±0.20	F_2 ♀♀	190	11.85±0.33	4.95±0.39	12.7
		Standard deviation			Standard deviation		
Wild first generation ♂♂	43	1.96±0.14	Tame first generation ♂♂	63	7.70±0.46	5.74±0.48	12.0
Wild first generation ♀♀	47	1.70±0.12	Tame first generation ♀♀	54	8.99±0.58	7.29±0.59	12.4
Wild first generation ♂♂	43	1.96±0.14	F_1 ♂♂	76	2.55±0.14	.59±0.20	3.0
Wild first generation ♀♀	47	1.70±0.12	F_1 ♀♀	88	2.82±0.14	1.12±0.18	6.2
Tame first generation ♂♂	63	7.70±0.46	F_1 ♂♂	76	2.55±0.14	5.15±0.48	10.7
Tame first generation ♀♀	54	8.99±0.58	F_1 ♀♀	88	2.82±0.14	6.17±0.60	10.3
F_1 ♂♂	76	2.55±0.14	F_2 ♂♂	175	7.59±0.27	5.04±0.30	16.8
F_1 ♀♀	88	2.82±0.14	F_2 ♀♀	190	6.77±0.23	3.95±0.26	15.2

TABLE 5

Distribution of wild and albino tame strains in the first, second and third trials.

SPEED IN SECONDS	MALES						FEMALES					
	WILD FIRST GENERATION			ALBINO TAME FIRST GENERATION			WILD FIRST GENERATION			ALBINO TAME FIRST GENERATION		
	FIRST TRIAL	SECOND TRIAL	THIRD TRIAL	FIRST TRIAL	SECOND TRIAL	THIRD TRIAL	FIRST TRIAL	SECOND TRIAL	THIRD TRIAL	FIRST TRIAL	SECOND TRIAL	THIRD TRIAL
3	1	2	4	..	..	..	2	10	14	..	..	..
4	3	9	9	..	..	..	8	16	12	..	..	..
5	5	6	8	..	..	..	12	9	9	..	..	..
6	5	6	10	..	..	..	11	3	7	..	..	1
7	4	10	7	..	..	..	5	6	3	..	..	1
8	7	4	3	..	.	..	3	1	..	..	1	1
9	5	3	..	..	..	..	4	..	1	..	1	2
10	6	1	1	..	1	2	1	..	..	1	..	..
11	3	1	..	..	..	5	..	1	..	..	1	6
12	1	..	1	..	1	3	..	..	1	1	2	2
13	..	1	..	..	1	4	1	..	..	..	1	3
14	1	..	..	..	3	4	..	1	..	..	4	2
15	1	..	..	..	1	1	..	..	..	..	3	1
16	1	..	..	..	6	4	..	..	..	..	3	3
17	..	..	..	..	3	4	..	..	..	..	3	3
18	..	..	..	1	1	..	..	..	..	..	3	3
19	..	..	..	1	5	2	..	..	..	..	3	6
20	..	..	..	1	2	2	..	..	..	1	4	..
21	..	..	..	1	3	4	..	..	..	..	2	1
22	..	..	..	2	2	1	..	..	..	..	2	1
23	..	..	..	2	3	1	..	..	..	2	2	..
24	..	..	..	1	2	..	..	..	..	..	..	3
25	..	..	..	2	..	..	..	..	..	1	2	..
26	..	..	..	1	..	..	..	..	..	2	..	1
27	..	..	..	2	2	..	..	..	..	..	..	..
28	..	..	..	1	1	1	..	..	..	..	..	..
29	..	..	..	3	2	1	..	..	..	1	1	..
30	..	..	..	..	..	..	..	..	..	1	1	1
31	..	..	..	3	..	..	..	..	..	4	..	..
32	..	..	..	2	..	..	..	..	..	1	..	..
33	..	..	..	1	..	..	..	..	..	1	..	..
34	..	..	..	2	..	..	..	..	..	1	..	..
35	..	..	..	1	..	..	..	..	..	5	..	..
36	..	..	..	2	..	..	..	..	..	..	..	..
37	..	..	..	..	..	..	..	..	..	1	..	..
38	..	..	..	2	..	..	..	..	..	..	1	..
39	..	..	..	..	..	..	..	..	..	2	..	..
40	..	..	..	3	..	..	..	..	..	..	..	..
41	..	..	..	..	..	..	..	..	..	5	..	..
42	..	..	..	..	..	..	..	..	..	1	..	..
43	..	..	..	1	..	..	..	..	..	2	1	..

TABLE 5 (continued)

SPEED IN SECONDS	MALES						FEMALES					
	WILD FIRST GENERATION			ALBINO TAME FIRST GENERATION			WILD FIRST GENERATION			ALBINO TAME FIRST GENERATION		
	FIRST TRIAL	SECOND TRIAL	THIRD TRIAL	FIRST TRIAL	SECOND TRIAL	THIRD TRIAL	FIRST TRIAL	SECOND TRIAL	THIRD TRIAL	FIRST TRIAL	SECOND TRIAL	THIRD TRIAL
44	..	..	..	1	..	..	..	..	..	..	..	..
45	..	..	..	1	..	..	..	..	..	..	..	..
46	..	..	..	1	..	..	..	..	..	2	..	..
47	..	..	..	..	..	..	..	..	..	2	..	..
50	..	..	..	1	..	..	..	..	..	..	..	..
55	..	..	..	..	..	..	..	..	..	1	..	..
61	..	..	..	..	..	..	..	..	..	1	..	..
66	..	..	..	..	..	..	..	..	..	1	..	..
68	..	..	..	..	..	..	..	..	..	1	..	..
Total	43	43	43	39	39	39	47	47	47	41	41	41
Mean	8.116		5.651	31.436		16.154	6.000		4.660	36.732		15.829
	6.302			19.385			4.936			18.976		
	±0.298		±0.189	±0.872		±0.502	±0.193		±0.174	±0.127		±0.565
	±0.221			±0.506			±0.207			±0.717		

TABLE 6

Comparison of the F_1's from reciprocal crosses in the runway test using the means of the populations.

PARENTS	MEANS OF F_1'S	
	MALES	FEMALES
Wild male×tame female	7.740±0.270	6.714±0.240
Tame male×wild female	7.120±0.167	7.360±0.374
Difference	0.620±0.317	0.646±0.444
Diff./P.E.	1.956	1.455

TABLE 7

Distribution in backcrosses in runway test average of three trials.

SPEED IN SECONDS	BACKCROSS TO WILD		BACKCROSS TO TAME	
	MALES	FEMALES	MALES	FEMALES
4	3	5	..	..
5	2	5	..	..
6	8	8	1	1
7	8	1	1	..
8	3	4	1	4
9	1	..	..	5
10	..	..	1	4
11	..	..	1	2
12	1	..	2	2
13	..	..	3	3
14	..	..	4	2
15	..	..	5	..
16	..	1	1	1
17	..	..	6	1
18	..	..	4	..
19	..	..	..	2
20	..	..	4	1
21	..	..	1	4
22	..	..	1	2
23	..	..	1	1
24	..	..	2	2
25	..	..	2	1
26	..	..	..	1
27	..	..	1	..
28	..	..	1	1
29	..	..	..	..
30	..	..	..	1
31	..	..	1	3
32	..	..	1	..
33	..	..	1	..
34	..	..	..	..
35	..	..	1	..
36	..	..	..	..
37	..	..	..	..
38	..	..	1	..
39	..	..	..	1
40	..	..	..	..
41	..	..	..	1
43	..	..	..	1
48	..	..	..	1
59	..	..	1	..
65	..	..	1	..
92	..	..	1	..
96	..	..	1	..
107	..	..	1	..
166	..	..	1	..
Total	26	24	54	48
Mean	6.577±0.217	6.167±0.334	27.389±2.589	18.729±0.993

TABLE 8

*Distribution of progeny from backcross (F_1 ♀ × *tame* ♂) and double backcross ([F_1 ♀ × *tame* ♂] ♀ × *tame* ♂) females mated to tame males. Runway test. Average of three trials.*

Sire	1352						1349			2090	1716					2356	2124	2355	
Speed	42.6						42.6			57.0	60.8					70.3	71.2	89.5	
Dam	1650	1649	1648	1659	1651	1829	1745	1738	1744	1822	1745	1743	1738	1742	1697	2102	2204	2371	2332
Speed	8.5	11.3	13.1	21.1	21.6	40.9	8.1	8.3	11.1	46.6	8.1	8.1	8.3	8.4	19.5	9.8	17.9	32.0	51.4
	8	22	21	22	28	26	15	12	19	..	..	47	30	32	20	12	40	59	49
Speed of	9	24	48	46	56	31	40	..	25	..	..	58	..	56	23	20	45	..	..
Male	11	31	53	..	61	..	46	..	33	..	..	100	..	67	24	21	61	..	..
Offspring	13	35	69	..	..	..	..	..	33	..	..	..	..	..	25	25	..	..	..
	22	37	..	..	..	..	..	..	..	..	..	..	..	..	27	31	..	..	..
	24	46	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	62	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Average	14.5	36.7	47.8	34.0	48.3	28.5	33.7	12.0	27.5			68.3	30.0	51.7	23.8	21.8	48.7	59.0	49.0
	7	9	18		22	9	11	8	15	28	14	21	9	18	20	24	29	22	24
	8	14	23		30	43	23	10	27	28	43	28	..	51	22	..	45	26	..
	9	17	26		35	..	34	14	28	36	..	34	..	..	34	..	57	33	..
Speed of	13	19	41		51	..	36	17	46	43	..	..	..	..	..	..	59	40	..
Female	14	25	..		..	..	58	..	..	..	..	..	..	..	..	..	..	..	..
Offspring	19	33	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	25	..	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	26	..	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	30	..	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	31	..	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	32	..	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	46	..	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	47	..	..		..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Average	23.6	19.5	27.0		34.5	26.0	32.4	12.2	29.0	33.8	28.5	27.7	9.0	34.5	25.3	24.0	47.5	30.2	24.0
Grand Average	20.7	28.8	37.4	34.0	40.4	27.2	32.9	12.2	28.2	33.8	28.5	48.0	19.5	44.8	24.4	22.2	48.0	36.0	36.5

NOTE: 2204, 2371 and 2332 are (F_1×tame)×tame. The others are F_1×tame.

TABLE 9

Results of selecting and mating F_2's and F_3's of different degrees of wildness and tameness. Runway test.

MEAN SPEED OF F_2 PARENTS	NUMBER OF YOUNG		RANGE		AVERAGE SPEED	
	MALES	FEMALES	MALES	FEMALES	MALES	FEMALES
4.2	4	4	4–5	4–6	4.8	4.8
5.8	5	6	7–11	5–9	8.4	7.0
6.2	4	6	7–44	8–37	20.8	21.2
9.1	2	3	7–27	5–6	17.0	5.7
9.5	14	14	9–18	6–36	13.9	13.5
11.4	13	25	11–44	5–31	20.8	17.9
20.4	1	5	8	10–15	8.0	13.4
26.1	..	1	..	50	..	50.0
Mean Speed of F_3 Parents						
4.3	8	3	4–9	6–8	5.6	7.3
18.6	9	7	13–42	11–23	22.8	17.3
31.6	10	6	19–50	23–38	31.7	30.8
40.5	7	3	38–205	42–56	71.3	48.7

TABLE 10

Results of selection on wild and tame stocks. Runway test. Average of three trials.

STOCK	GENERATION	MEAN	GENERATION	MEAN	DIFFERENCE	D/P.E.
Wild ♂♂	1	6.698±0.202	2	6.897±0.097	+0.199±0.224	0.9
Wild ♂♂	1	6.698±0.202	3	6.421±0.377	−0.277±0.428	0.6
Wild ♂♂	1	6.698±0.202	4*	5.000±0.377	−1.698±0.394	4.3
Wild ♂♂	2	6.897±0.097	3	6.421±0.377	−0.476±0.389	1.2
Wild ♂♂	2	6.897±0.097	4	5.000±0.337	−1.897±0.351	5.4
Wild ♂♂	3	6.421±0.377	4	5.000±0.337	−1.421±0.506	2.8
Wild ♀♀	1	5.277±0.167	2	5.588±0.381	+0.311±0.416	0.7
Wild ♀♀	1	5.277±0.167	3	5.250±0.163	−0.027±0.233	0.1
Wild ♀♀	1	5.277±0.167	4	5.857±0.318	+0.580±0.359	1.6
Wild ♀♀	2	5.588±0.381	3	5.250±0.163	−0.338±0.415	0.8
Wild ♀♀	2	5.588±0.381	4	5.857±0.318	−0.269±0.496	0.5
Wild ♀♀	3	5.250±0.163	4	5.857±0.318	+0.607±0.358	1.7
Tame ♂♂	1	24.492±0.654	2	39.562±1.736	+15.070±1.855	8.1
Tame ♂♂	1	24.492±0.654	3	45.500±2.849	+21.008±2.923	7.2
Tame ♂♂	1	24.492±0.654	4	72.000±3.969	+47.508±4.022	11.8
Tame ♂♂	2	39.562±1.736	3	45.500±2.849	+5.938±3.336	1.8
Tame ♂♂	2	39.562±1.736	4	72.000±3.969	+32.438±4.332	7.5
Tame ♂♂	3	45.500±2.849	4	72.000±3.969	+26.500±4.886	5.4
Tame ♀♀	1	25.352±0.825	2	34.188±1.782	+8.836±1.964	4.5
Tame ♀♀	1	25.352±0.825	3	44.000±3.382	+18.648±3.481	5.4
Tame ♀♀	1	25.352±0.825	4	54.375±2.696	+29.023±2.819	10.3
Tame ♀♀	2	34.188±1.782	3	44.000±3.382	+9.812±3.822	2.6
Tame ♀♀	2	34.188±1.782	4	54.375±2.696	+20.187±3.231	6.2
Tame ♀♀	3	44.000±3.382	4	54.375±2.696	+10.375±4.324	2.4

* The fourth generation wild males was very small, consisting of only four individuals.

TABLE 11

Analysis of the F_2 population for indications of linkage between wildness and albinism, agouti, brown, pink eye and short ear.

CHARACTER	MALES		FEMALES	
	NUMBER	MEAN	NUMBER	MEAN
Colored	148	11.885±0.300	160	11.012±0.316
Albino	27	18.778±1.706	30	16.333±1.099
Difference		+6.893±1.732		+5.321±1.144
Diff./P.E.		4.0		4.7
Agouti	118	11.941±0.338	134	10.463±0.309
Non Agouti	30	11.667±0.650	26	13.846±1.046
Difference		−0.274±0.732		+3.383±1.090
Diff./P.E.		0.4		3.1
Black	134	11.403±0.280	134	10.381±0.303
Brown	14	16.500±1.458	26	14.269±1.065
Difference		+5.097±1.484		+3.888±1.108
Diff./P.E.		3.4		3.5
Dark eye	127	11.268±0.293	135	9.822±0.268
Pink eye	21	15.619±0.993	25	17.440±1.056
Difference		+4.351±1.035		+7.618±1.090
Diff./P.E.		4.2		7.0
Normal ear	134	11.881±0.321	150	10.753±0.317
Short ear	14	11.929±0.766	10	14.900±1.527
Difference		+0.048±0.831		+4.147±1.559
Diff./P.E.		0.1		2.7

NOTE: The mice were not separated into the strains from which they came. Thus the recessive genes for some characters could not have occurred in the whole population.

TABLE 12

Analysis of the population of the backcross to the tame by strains for indications of linkage between wildness and albinism, agouti, brown and pink eye.

CHARACTER	MALES		FEMALES	
	NUMBER	MEAN	NUMBER	MEAN
MICE FROM ALBINO AND WILD STRAINS*				
Colored	21	23.952±3.166	13	18.077±1.636
Albino	18	33.278±5.838	18	16.056±1.591
Difference		+9.326±6.641		−2.021±2.282
Diff./P.E.		1.4		0.9
Agouti†	15	24.067±4.071	12	15.500±1.935
Non Agouti	6	23.667±4.388	6	17.167±2.770
Difference		−0.400±5.986		+1.667±3.379
Diff./P.E.		0.7		0.5
MICE FROM PINK EYED BROWN AND WILD STRAINS				
Black	5	18.600±1.497	5	15.400±1.765
Brown	7	18.143±1.621	6	29.833±3.394
Difference		−0.457±2.207		+14.433±3.825
Diff./P.E.		0.2		3.8
Agouti	5	14.200±1.327	5	26.600±4.457
Non Agouti	7	21.286±1.232	6	20.500±2.405
Difference		+7.086±1.811		−6.100±5.645
Diff./P.E.		3.9		1.1
Dark eye	6	19.500±1.366	4	20.500±5.446
Pink eye	6	17.167±1.752	7	24.857±2.278
Difference		−2.333±2.222		+4.357±5.903
Diff./P.E.		1.0		0.7

* This does not include mice from F_1 ♀ 544 which was a Detlefsen mutant, apparently introduced through the wild strain.

† Apparently only part of the albino stock carried non agouti.

FURTHER OBSERVATIONS ON *LYTHRUM SALICARIA*

E. M. EAST

Harvard University, Cambridge, Massachusetts

Received October 15, 1931

In an earlier paper (EAST 1927), it was shown that the inheritance of heterostyly in *Lythrum salicaria* can not be described in a complete and satisfactory manner by the hypothesis that mid-styled plants are conditioned by a factor M, that short-styled plants are conditioned by a factor A which is epistatic to M, and that long-styled plants are conditioned by the action of the two recessive allelomorphs am. It is true that Mrs. BARLOW (1923), Miss VON UBISCH (1925), and I have studied crosses where the results fit the assumption that only these two factors are involved; but Mrs. BARLOW has also studied several crosses in which the results could not be interpreted so simply; and later, working with plants which came from Mrs. BARLOW'S cultures, I have observed a similar complexity of behavior. There is no difficulty in accounting for Shorts. Shorts, in the experience of all workers on Lythrum, are conditioned by the factor A, which is always found in the heterozygous condition. And the factors A and a are distributed in the regular orthodox manner. The disturbing feature of the situation is the behavior of certain Mids which consistently throw about 10 percent more "Long" gametes than would be expected on the two-factor hypothesis. For example, as I reported earlier, Longs crossed with pollen from certain "homozygous" Mids gave 19 Longs to 191 Mids, while these same "homozygous" Mids, crossed with pollen from Longs, gave 9 Longs to 121 Mids. Additional data from similar crosses now make the total figures read as follows: "homozygous" Mids used as males on Longs have given 50 Longs to 438 Mids, and Longs used as males on "homozygous" Mids have given 22 Longs and 200 Mids. Thus about 10 percent of both the megaspores and the microspores of these so-called "homozygous" Mids carried the factors for long-style.

These facts, together with consistent data from other crosses, were interpreted (EAST 1927) by assuming that the particular Mids with which we were dealing owed their existence to two duplicate linked factors M_a and M_b, which have a lethal effect if in the homozygous condition and show a crossover value of 10 percent. Under such an assumption four types of Mids may exist, namely

(1) $\frac{M_aM_b}{m_am_b}$, (2) $\frac{M_am_b}{m_aM_b}$, (3) $\frac{M_am_b}{m_am_b}$, and (4) $\frac{m_aM_b}{m_am_b}$.

Type 2 is the "homozygous" Mid under consideration. Types 3 and 4 contain only one factor for Mid and should show no complexity in their behavior.

A reference to the data reported in my earlier paper will show the reader that nearly all crosses involving plants other than certain descendants of those which were received from Mrs. BARLOW conformed in their behavior to the simple two-factor hypothesis. In fact, the way that the behavior of the majority of plants fits this idea is rather remarkable, considering the fact that we are dealing with a species where the plants are highly self-sterile, where plants bearing flowers of like forms are highly cross-sterile, where the percentage of abortive pollen is large, and where the germination of the seeds is poor. From the consistency of the results under the conditions which obtain, therefore, one may conclude that ordinarily pollen and seed elimination are not sufficiently selective to render unsatisfactory the simple two-factor interpretation of heterostyly. We have only the behavior of the aberrant Mids to explain. Altogether there were five of these so-called "homozygous" Mids, though only two were tested in numerous crosses. Their behavior was consistent with the balanced lethal hypothesis. No critical proof of the balanced lethal condition has been offered, however; it is merely a plausible theory. And it will probably remain only a plausible theory, for critical evidence could be offered only by testing the genetic constitutions of such a large number of plants that the game would hardly be worth the candle.

Since the earlier data were reported, I have been able to make a number of additional observations, some of which appear to be worthy of record. (1) Attempts at a cytological analysis of Lythrum material have resulted in failure. The species is highly unsatisfactory for such work. The chromosomes, of which there are apparently 24 pairs in each form, are very small, rendering it practically impossible to say whether the aberrant Mids are different from ordinary Mids. (2) It is impossible to say whether the families which should show high percentages of non-viable zygotes on the balanced lethal hypothesis actually do differentiate themselves from families where no lethal combinations are present. A large number of germination tests were made; but in all types of combination, with one exception, the percent of seeds germinating varied from 7 to 60. The one exception is a set of four crosses where homozygous Mids having presumably but one factor were involved. Here the germination was rather high, being in fact between 65 and 80 percent. Perhaps the fact is significant. (3) Results from some 40 additional families where presumably only one Mid factor was involved show that in such cases behavior is normal. I will report only

the results from families where the germination of seeds was high and the resulting populations were large enough to give some confidence in the evidence.

Plants from families where but one Mid factor was suspected were selfed. Selfing is difficult, of course, and the number of plants obtained is usually small; but 14 Mids gave results which indicated that their constitution was of this type. One of these Mids was crossed four times with Longs. The data are found in table 1. These families are all somewhat larger than one usually obtains; and, as I have already remarked, the germination of the seeds from which they came was between 65 and 80 percent, showing that there was little possibility of a selective elimination of zygotes. *The results show that homozygous Mids can be obtained which throw no gametes carrying Long.*

TABLE 1

Results from crossing Longs with homozygous Mids having but one factor.

NUMBER	ORIGIN	CROSS	RESULT		
			L	*M*	*S*
1401	*LC*×1208–21	*L*×*M*	0	162	1
1402	1208–21×*LC*	*M*×*L*	0	151	0
1403	1208–21×*LB*	*M*×*L*	0	95	0
1315	1208–21×1202–49	*M*×*L*	1	99	0

In addition to these cases, twenty-one crosses were made between Longs and heterozygous Mids supposed to contain but one factor. Most of the resulting populations were too small to give significant figures, and may be disregarded. There were eight families, however, where the numbers are reasonably large (table 2). Families 1501, 1505, 1506, and 1509 are

TABLE 2

Results from crossing Longs with heterozygous Mids having but one factor.

NUMBER	ORIGIN	CROSS	RESULT		
			S	*M*	*L*
1501	*LA*×*MA*	*L*×*M*	1	58	58
1505	1401–45×1408–43	*M*×*L*	0	61	66
1506	1408–43×1401–45	*L*×*M*	0	42	56
1509	1311– 2×*LA*	*M*×*L*	1	151	152
1516	*LA*×1311–20	*L*×*M*	0	29	16
1518	1311–69×*LA*	*M*×*L*	0	25	18
1503	1401– 1×1408–43	*M*×*L*	0	31	9
1504	1408–43×1401–1	*L*×*M*	1	127	25

clearly good one to one ratios. They total 332 Longs to 312 Mids. Families 1516 and 1518 show an excess of Mids, since there are 54 Mids to 34 Longs; but this excess is not enough to make it probable that we are dealing with additional variables. In Families 1503 and 1504, coming from reciprocal matings, there is, on the other hand, a sufficient excess of Mids to make one suspect some complicating factor or factors. The figures are 158 Mids to 34 Longs. I shall speak of this matter again a little later.

From these data and from the other published records (one may pick out obvious cases in the papers of Mrs. BARLOW, Miss VON UBISCH, and EAST), it is clear that the Mids of *Lythrum salicaria* are ordinarily conditioned by a single factor that behaves in the normal manner.

It is only necessary, then, to account for the behavior of the aberrant type of Mid. I have seen five plants definitely of this type. I suspect that some six or eight others that I have observed and some three or four of those that Mrs. BARLOW has observed, have the same constitution. Thus all of those found are from samples of a single stock. We do not know, therefore, whether these aberrant Mids are rare in nature or not. Miss VON UBISCH has not observed them in her German material, and apparently no such type has been observed in the relatively few plants of other trimorphic species that have been investigated. At all events, they are not common.

I stated, in 1927, that Mids having both representatives of the duplicate factors postulated to account for the aberrant individuals ought to be more frequent in nature than those having but one. This was simply a slip of the pen, as a careful reading of the paper will show that what I had in mind at that time was the relative frequency of individuals having duplicate genes in the populations under consideration, these populations being the progeny of plants assumed to have duplicate genes.

As I have already stated, the data concerning the behavior of these aberrant Mids are not of a type to establish the duplicate factor hypothesis. Nevertheless, there are reasons for believing that this hypothesis is the most plausible of those which will fit the facts. One can not utilize any system of independent multiple factors, of linkage without balanced lethals, or of multiple allelomorphs. One can also rule out nearly all of the common forms of reduplication, inversion, and similar types of abnormal chromosome distribution. They will not fit the facts.

It is possible to account for the *origin* of the duplicate linked factors $M_a - M_b$ at 10 units distance from each other by one of the rarer forms of abnormal chromosome distribution; but when this condition has once arisen, normal chromosome distribution is required if the segregations

actually found are to be explained satisfactorily. Duplicate linked factors would be produced if a piece of a chromosome containing the gene *M* were inserted in an homologous chromosome at the proper point. The mere apposition and continued union of two homologous chromosomes, on the other hand, would not fit the requirements. Attachment at homologous ends presumably would lead to a situation similar to that in the attached-X stock of Drosophila; attachment at non-homologous ends probably would result in crossover values higher than 10 percent. Such speculations are unnecessary, however, for the origin of the duplicate linked factors postulated in the balanced lethal hypothesis is not in question. Genes having the same or similar functions are known to exist in various species. So far as I know, no linked duplicate genes have been described; but it is not unreasonable to assume that they may exist.

Unless I have overlooked some of the facts, then, there are only three ways of explaining the behavior of our aberrant Mids, non-disjunction, mutation, and balanced lethals.

If, because of unequal chromosome distribution, a trisomic condition arose in which there were factors *M–M–m*, then unless unknown variables were present to increase the complexity of the situation, the gametes formed would be *MM*, *Mm*, *Mm*, *M*, *M* and *m*. Self-fertilization of this type of homozygous Mid would give 1 Long to 35 Mids. Crossed with Longs, there would be 1 Long to 5 Mids. This condition may possibly be what we have in Families 1503 and 1504 of table 2. I am not inclined to favor this hypothesis, however, for a reason beyond that of OCCAM'S test of the validity of hypotheses. In order to obtain the results actually found, other than those in Families 1503 and 1504, one would have to suppose that the non-disjunction *Mm–M* occurs about three times as frequently as the non-disjunction *MM–m*. One can not deny the possibility that such an assumption accords with the facts. It has been used, I believe, by other geneticists to account for abnormal ratios. Yet evidence is accumulating that non-disjunction results from loose-pairing or non-pairing of chromosomes, and is, therefore, just the opposite of what the term implies. It is a mechanism that is not exact and produces ratios that are not very consistent. This does not accord with our experience where over 50 families involving three plants of the aberrant "homozygous" Mid type have given very consistent results. The consistency of these results—that is to say the uniformity with which these aberrant "homozygous" Mids produce 10 percent of Long gametes—appears to demand a mechanism at least as exact as crossing over, for crossing over itself is a more variable procedure than most biological processes.

The second possibility of interpreting the behavior of these interesting Mids is mutation. Two different correspondents have called my attention to the fact that the simplest interpretation of our data is to assume that a factor *M* conditioning Mid mutates to *m* about ten times per 100 gametes. This hypothesis had been considered before the publication of my 1927 paper but had been abandoned for the following reasons. Unless it be assumed that the aberrant type of Mid is an ephemeral condition which Mrs. BARLOW happened to discover at exactly the right moment, a convincing interpretation of its genetic behavior should provide for survival. Since there is no evidence that *m* ever mutates to *M* at a higher rate of frequency than is to be expected for any chance mutation and since there is no evidence that natural selection favors Mids, it is obvious that such a high rate of mutation from *M* to *m* would soon result in the elimination of all mid-styled plants. Now no such process appears to be going on. On the contrary, plants of the three style-lengths have about the same frequency in nature. Perhaps there is some deficiency of Shorts, but Longs and Mids have been found in approximately equal numbers in the various censuses that have been made. These censuses are as follows (see VON UBISCH 1925).

DARWIN, in England, 148 Longs: 135 Mids: 110 Shorts; KEPPERT, in Germany, 64 Longs: 36 Mids: 44 Shorts; VON UBISCH, in Germany, 161 Longs: 191 Mids: 109 Shorts; EAST, in Massachusetts, U.S.A., 134 Longs: 151 Mids: 122 Shorts.

Both the two-factor hypothesis and the balanced lethal hypothesis satisfy the requirement that plants of the three forms come to equilibrium at approximately equal frequency, if self-sterility, cross-sterility of like forms, random mating, and random survival are assumed. For example, with 48 plants of which 12 are *aamm*, 8 are *aaMm*, 4 are *aaMM*, 3 are *Aamm*, 6 are *AaMm*, and 3 are *AaMM*—giving equal numbers of Longs, Mids, and Shorts—after 10 generations of random mating the figures are 11.42 *aamm*, 11.44 *aaMm*, 1.04 *aaMM*, 6.03 *Aamm*, 5.02 *AaMm*, and 1.04 *AaMM*.

Similar results flow from the balanced lethal hypothesis. Three generations of random matings are shown in table 3, assuming that types $\frac{M_a m_b}{M_a m_b}$ and $\frac{m_a M_b}{m_a M_b}$ are viable, but not types $\frac{M_a M_b}{M_a m_b}$, $\frac{M_a M_b}{m_a M_b}$ or $\frac{M_a m_b}{M_a M_b}$. As the calculations are rather tedious, I have not carried the problem any further.

It is sufficient to see that there is a general trend toward equilibrium when the three forms of plants are approximately equal, and to note that the single factor Mids will be about nine times as numerous as the duplicate factor Mids.

TABLE 3

Results of random matings of different types of Lythrum salicaria under the balanced lethal hypothesis crossovers 10 percent.

CONSTITUTION	FREQUENCY IN GENERATION NUMBER			
	1	2	3	4
$aamambmamb$	16	11.153	13.685	14.306
Total Longs	16	11.153	13.685	14.306
$aaM_am_bm_am_b$	4	8.564	7.408	7.459
$aam_aM_bm_am_b$	4	8.564	7.408	7.459
$aaM_am_bM_am_b$	2	0.479	0.465	0.397
$aam_aM_bm_aM_b$	2	0.479	0.465	0.397
$aaM_aM_bm_am_b$	2	1.981	1.317	0.998
$aaM_am_bm_aM_b$	2	0.959	0.930	0.793
Total Mids	16	21.026	17.993	17.503
$Aam_am_bm_am_b$	6	6.040	6.744	7.319
$AaM_am_bm_am_b$	2	3.451	3.543	3.419
$Aam_aM_bm_am_b$	2	3.451	3.543	3.419
$AaM_am_bM_am_b$	1	0.479	0.465	0.397
$Aam_aM_bm_aM_b$	1	0.479	0.465	0.397
$AaM_aM_bm_am_b$	2	0.959	0.631	0.443
$AaM_am_bm_aM_b$	2	0.959	0.930	0.799
Total Shorts	16	15.818	16.321	16.193

Another argument which makes the "mutation" theory unworthy of serious consideration derives from the fact that there are Mids, originating from the same stock as the aberrant Mids, which throw no Longs. It would be strange to assume that the factor *M* is mutating rapidly in some individuals and is constant in others.

There is left the balanced lethal interpretation of the behavior of the aberrant Mids. The evidence taken as a whole, including that presented earlier (EAST 1927), makes it the most plausible theory to hold, though I do not claim that it is established beyond reasonable doubts.

LITERATURE CITED

BARLOW, N., 1913 Preliminary note on heterostylism in Oxalis and Lythrum. J. Genet. **6**: 53–65.
1923 Inheritance of the three forms in trimorphic species. J. Genet. **13**: 133–146.
EAST, E. M., 1927 The inheritance of heterostyly in *Lythrum salicaria*. Genetics **12**: 393–414.
UBISCH, G. VON, 1921 Zur Genetik der trimorphen Heterostylie sowie einige Bemerkungen zur dimorphen Heterostylie. Biol. Zbl. **41**: 88–96.
1923 Versuche über Vererbung und Fertilität bei Heterostylie und Blütenfüllung. Z. Bot. **15**: 193–232.
1925 Genetisch-physiologische Analyse der Heterostylie. Bibl. genet. **2**: 287–342.

METHOD OF MEASURING LINKAGE IN HUMAN GENETICS; WITH SPECIAL REFERENCE TO BLOOD GROUPS

ALEXANDER S. WIENER
Jewish Hospital of Brooklyn, New York

Received October 16, 1931

TABLE OF CONTENTS

	PAGE
INTRODUCTION	335
Are the agglutinogens *M* and *N* linked to *A* and *B*?	337
Derivation of formula for Q	346
Derivation of formula for standard error of Q	347
SUMMARY	349
LITERATURE CITED	350

INTRODUCTION

Whereas much has been learned regarding the genetics of many plants and animals in the past thirty years (the work of MORGAN and his coworkers on the *Drosophila melanogaster* is particularly notable), comparatively little progress has been made in man. The main reason for this is that the problems of human inheritance must be solved by somewhat different methods than those employed in plants and animals. Direct experimentation is obviously impossible, and data must often be accumulated by the less exact method of analyzing pedigrees and family histories. Studies in linkage, particularly, offer difficulties in human genetics. In plants or animals, when one wishes to determine whether or not any two mutations are linked in their heredity, all that is necessary is to cross two strains which possess the mutations in question, and examine the progeny for one or two generations. In man, however, data must be accumulated from crosses which are already made. Thus, if it were desired to study the linkage of white forelock and polydactylism, a search would have to be made for families presenting both of these anomalies. Since both of these anomalies are of infrequent occurrence, families possessing both traits at the same time must be exceedingly rare. Furthermore, because of the small size of human families, it is necessary to examine many families before any conclusions can be drawn, so that studies of this nature become practically impossible.

It is less difficult, of course, to determine whether or not sex-linkage is present, and at least fifteen human mutations have been found to be sex-

linked. The more important of the sex-linked characters are haemophilia, red-green color-blindness, optic atrophy (Leber's disease), and night blindness. Recently, an attempt was made by DAVENPORT (1930) to find families possessing two sex-linked traits at the same time, in order to determine the amount of crossing over between the sex-linked factors. Such families were found to be so rare, that no definite conclusions could be drawn. A remarkable family has been described by MADLENER (1928), however, in which a man who is both haemophilic and color-blind has a son who is haemophilic, two grandsons who are haemophilic and color-blind, and a great grandson who is haemophilic, but too young yet to determine whether or not color-blindness is present.

The discovery of the LANDSTEINER blood groups and the mechanism of their heredity opened the field for further studies in human linkage. Because of the simple heredity of the blood groups, and because of their universal distribution, it is possible now, by an examination of a sufficient number of families, to determine whether or not linkage exists between any hereditary trait and the blood groups. Although a number of such studies have already been made, no case of linkage has yet been found. Thus SNYDER (1929, 1931) found no evidence of linkage between polydactylism, telangiectasis, various eye anomalies and the blood groups; and LEVINE (1926) found no evidence of linkage between atopic hypersensitiveness and the blood groups. Because of the small number of such studies made thus far as compared with the number of chromosomes (twenty-four pairs), it is not surprising that not a single case of human linkage has been found, except for the cases of sex-linkage already mentioned. KUBANYI (1931) has determined the blood groups of members of haemophilic families, in order to determine the presence or absence of linkage. KUBANYI'S work, it would seem, is unnecessary, for haemophilia is a sex-linked trait, whereas the blood groups are not, so that linkage between these two characters is hardly possible.

More recently, two additional agglutinogens, *M* and *N*, have been described by LANDSTEINER and LEVINE (1928), and these agglutinogens also have a simple Mendelian heredity. From an inspection of their family material, LANDSTEINER and LEVINE concluded that the agglutinogens *M* and *N* are most likely inherited independently of the agglutinogens *A* and *B*. This discovery has therefore opened up further possibilities for linkage studies in human heredity.

Accordingly there are now three out of the total of twenty-four pairs of chromosomes marked by simple hereditary factors: one by sex, the second by the LANDSTEINER blood groups, and the third by the agglutinogens *M*

and *N* of LANDSTEINER and LEVINE. Whereas the color of eyes, hair and skin, finger-prints, etc. also have a universal distribution, their heredity is complex and not yet completely solved, so that they cannot be used as a basis for linkage studies. On the other hand, such characters as polydactylism, brachydactyly, zygodactyly, 'lobster claw,' etc., which have a simple heredity, are of too rare occurrence to be of much value. It seems obvious that conclusions regarding linkage can only be drawn from family material, and not from determinations of correlations between traits in a population. As is well known, there is a close association between hair-color and the color of the eyes. Thus, blue eyes are most commonly found associated with light brown or flaxen hair, and brown eyes with dark hair. Blue eyes combined with dark hair, and brown eyes combined with light hair are less commonly found. HAECKER (1925) suggests an explanation for this association on the ground that the genes for hair-color and eye-color are in the same chromosome. Then the "disharmonic phaenotypic combinations might be explained by crossing over." As DAVENPORT (1927) points out, however, HAECKER'S interpretation of "crossing over" is not that of students of Drosophila. It seems more likely to the present author that the combinations of blue eyes with light hair and dark eyes with dark hair may have arisen from two different races living in different parts of the world. The less frequent combinations, light hair with brown eyes, and dark hair with blue eyes, would then represent hybrids resulting from crosses between individuals of these two races.

In the studies in human heredity that have thus far been made, the presence or absence of linkage was determined from an inspection of the family material. The need for an exact mathematical method of measuring linkage, applicable in human genetics, is the cause of this paper, as by a mere inspection of the families a loose linkage with much crossing over would be overlooked. BERNSTEIN (1931) has already worked out a method which he applied to the question of linkage between the agglutinogens *M* and *N* and the agglutinogens *A* and *B*, and arrived at the conclusion that *M* and *N* are most likely inherited independently of *A* and *B*. We have also derived a method that can be used wherever the question of linkage arises in human genetics. By way of illustration we shall apply the method to the question of linkage of the agglutinogens *M* and *N* to *A* and *B*.

ARE THE AGGLUTINOGENS *M* AND *N* LINKED TO *A* AND *B*?

The only type of families in which the presence of linkage can make any difference in the types of the offspring is that in which one of the parents is doubly heterozygous. That is, one of the parents must belong to one of

the following types: $AB++$, A_h++, or B_h++. The letters O, A, B, AB represent the blood groups; the first $+$ or $-$ sign the reaction for M, and the second $+$ or $-$ sign the reaction for N. We use the subscript "h" to represent the heterozygous genotype of groups A and B, and the subscript "p" to represent the homozygous or pure genotype. If there are group O children from group A parents, such parents are necessarily heterozygous. In table 1 the offspring of all matings involving these three types of parents are given, and the offspring are subdivided into three classes: "linked," "crossover," and "indeterminate" types. Thus, in the cross $AB++ \times O++$, if the genotype of the $AB++$ parent is $(AM)(BN)$, the linked gametes from that parent are (AM) and (BN), and the crossover gametes are (AN) and (BM). The $O++$ parent produces (RM) and (RN) gametes in equal numbers. The linked zygotes, therefore, are $(AM)(RM)$, $(AM)(RN)$, $(BN)(RM)$, and $(BN)(RN)$, and the crossover zygotes are $(AN)(RM)$, $(AN)(RN)$, $(BM)(RM)$, and $(BM)(RN)$. Hence, the linked types are $A+-$ and $B-+$, and the crossover types are $A-+$ and $B+-$; whereas the types $A++$ and $B++$ might be either linked or crossovers and are therefore placed in the column headed "indeterminate." The remainder of the table was worked out in a similar manner.

If we could determine whether an individual of type $AB++$ belongs to genotype $(AM)(BN)$ or $(AN)(BM)$, and similarly for the types A_h++ and B_h++, it would be comparatively simple to determine the crossover value. This could often be done by determining the types of the grandparents (see table 2). Thus, an individual of type $AB++$ with parents $A+-$ and $B++$, can only belong to genotype $(AM)(BN)$; such an individual with parents $A-+$ and $B++$ must belong to genotype $(AN)(BM)$; but if the parents are $A++$ and $B++$, the genotype is indeterminate. Unfortunately, however, it is very difficult, for obvious reasons, to obtain three generations in studies on human heredity. In the studies on the heredity of the agglutinogens A, B, M, and N, that have appeared thus far, the grandparents were not examined. It is therefore necessary to devise a method of measuring linkage based on data from only two generations and involving only comparatively small families.

In table 3 is presented an analysis of all the data that have thus far been accumulated on the heredity of the agglutinogens A, B, M, and N, that can be used for the purpose of measuring linkage. The second series of LANDSTEINER and LEVINE has not been published yet but was made available to us through the courtesy of the authors. The table only includes matings involving the types $AB++$, A_h++, and B_h++. With the aid of table 1 the offspring of these crosses were subdivided into three

TABLE 1

CROSS	LINKED	CROSSOVERS	INDETERMINATE
$AB++\times O++$	$A+-\ B-+$	$A-+\ B+-$	$A++\ B++$
$AB++\times O+-$	$A+-\ B++$	$A++\ B+-$	..
$AB++\times O-+$	$A++\ B-+$	$A-+\ B++$	..
$AB++\times A_p++$	$AB-+\ A+-$	$AB+-\ A-+$	$AB++\ A++$
$AB++\times A_p+-$	$AB++\ A+-$	$AB+-\ A++$	..
$AB++\times A_h+-$	$AB++\ A+-\ B++$	$AB+-\ A++$ $B+-$	..
$AB++\times A_p-+$	$AB++\ A-+$	$AB-+\ A++$	..
$AB++\times A_h-+$	$AB++\ A-+\ B++$	$AB-+\ A++$ $B-+$	..
$AB++\times B_p++$	$AB-+\ B+-$	$AB+-\ B-+$	$AB++\ B++$
$AB++\times B_p+-$	$AB++\ B+-$	$AB+-\ B++$	..
$AB++\times B_h+-$	$AB++\ A++\ B+-$	$AB+-\ A+-$ $B++$	..
$AB++\times B_p-+$	$AB++\ B-+$	$AB-+\ B++$	..
$AB++\times B_h-+$	$AB++\ A++\ B-+$	$AB-+\ A-+$ $B++$	..
$AB++\times AB+-$	$A+-\ B++$	$A++\ B+-$	$AB++\ AB+-$
$AB++\times AB-+$	$A-+\ B++$	$A++\ B-+$	$AB++\ AB-+$
$A_h++\times O++$	$A+-\ O-+$	$A-+\ O+-$	$A++\ O++$
$A_h++\times O+-$	$A+-\ O++$	$A++\ O+-$	..
$A_h++\times O-+$	$A-+\ O++$	$A++\ O-+$	..
$A_h++\times A_h+-$	$O++$	$O+-$	$A++\ A+-$
$A_h++\times A_h-+$	$O++$	$O-+$	$A++\ A-+$
$A_h++\times B_p++$	$AB+-\ B-+$	$AB-+\ B+-$	$AB++\ B++$
$A_h++\times B_p+-$	$AB+-\ B++$	$AB++\ B+-$	..
$A_h++\times B_h+-$	$AB+-\ A+-\ B++$ $O++$	$AB++\ A++$ $B+-\ O+-$	..
$A_h++\times B_p-+$	$AB-+\ B++$	$AB++\ B-+$	..
$A_h++\times B_h-+$	$AB-+\ A-+\ B++$ $O++$	$AB++\ A++$ $B-+\ O-+$	..
$A_h++\times AB+-$	$AB+-\ B++$	$AB++\ B+-$	$A++\ A+-$
$A_h++\times AB-+$	$AB-+\ B++$	$AB++\ B-+$	$A++\ A-+$
$B_h++\times O++$	$B+-\ O-+$	$B-+\ O+-$	$B++\ O++$
$B_h++\times O+-$	$B+-\ O++$	$B++\ O+-$	..
$B_h++\times O-+$	$B-+\ O++$	$B++\ O-+$	..
$B_h++\times A_p++$	$AB+-\ A-+$	$AB-+\ A+-$	$AB++\ A++$
$B_h++\times A_p+-$	$AB+-\ A++$	$AB++\ A+-$	..
$B_h++\times A_h+-$	$AB+-\ A++\ B+-$ $O++$	$AB++\ A+-$ $B++\ O+-$	..
$B_h++\times A_p-+$	$AB-+\ A++$	$AB++\ A-+$	..
$B_h++\times A_h-+$	$AB-+\ A++\ B-+$ $O++$	$AB++\ A-+$ $B++\ O-+$	..
$B_h++\times B_h+-$	$O++$	$O+-$	$B++\ B+-$
$B_h++\times B_h-+$	$O++$	$O-+$	$B++\ B-+$
$B_h++\times AB+-$	$AB+-\ A++$	$AB++\ A+-$	$B++\ B+-$
$B_h++\times AB-+$	$AB-+\ A++$	$AB++\ A-+$	$B++\ B-+$

NOTE: In this table the parents of types $AB++$, A_h++, and B_h++ are assumed to be of linkage types (AM) (BN), (AM) (RN), and (BM) (RN), respectively. For the linkage types (AN) (BM), (AN) (RM), and (BN) (RM) the same results hold except that the columns headed "linked" and "crossovers" are interchanged.

TABLE 2

PHENOTYPE	GENOTYPE	WILL BE PRODUCED BY CROSSES			
$AB{+}{+}$	$(AM)\,(BN)$	$A{+}{-} \times \begin{Bmatrix} B{+}{+} \\ B{-}{+} \\ AB{+}{+} \\ AB{-}{+} \end{Bmatrix}$	$A{+}{+} \times \begin{Bmatrix} B{-}{+} \\ AB{-}{+} \end{Bmatrix}$	$AB{+}{-} \times \begin{Bmatrix} B{-}{+} \\ B{+}{+} \end{Bmatrix}$	$AB{+}{+} \times B{-}{+}$
	$(AN)\,(BM)$	$A{-}{+} \times \begin{Bmatrix} B{+}{+} \\ B{+}{-} \\ AB{+}{+} \\ AB{+}{-} \end{Bmatrix}$	$A{+}{+} \times \begin{Bmatrix} B{+}{-} \\ AB{+}{-} \end{Bmatrix}$	$AB{-}{+} \times \begin{Bmatrix} B{+}{-} \\ B{+}{+} \end{Bmatrix}$	$AB{+}{+} \times B{+}{-}$
	Indeterminate	$\left.\begin{matrix} A{+}{+} \\ AB{+}{+} \end{matrix}\right\} \times \begin{Bmatrix} B{+}{+} \\ AB{+}{+} \end{Bmatrix}$	$AB{+}{+} \times \begin{Bmatrix} AB{-}{+} \\ AB{+}{-} \end{Bmatrix}$	$AB{-}{+} \times AB{+}{-}$	
$A{+}{+}$	$(AM)\,(RN)$	$\left.\begin{matrix} A{+}{-} \\ AB{+}{-} \end{matrix}\right\} \times \begin{Bmatrix} O{-}{+} \\ O{+}{+} \\ B{-}{+} \\ B{+}{+} \end{Bmatrix}$	$\left.\begin{matrix} A{+}{+} \\ AB{+}{+} \end{matrix}\right\} \times \begin{Bmatrix} O{-}{+} \\ B{-}{+} \end{Bmatrix}$		
	$(AN)\,(RM)$	$\left.\begin{matrix} A{-}{+} \\ AB{-}{+} \end{matrix}\right\} \times \begin{Bmatrix} O{+}{-} \\ O{+}{+} \\ B{+}{-} \\ B{+}{+} \end{Bmatrix}$	$\left.\begin{matrix} A{+}{+} \\ AB{+}{+} \end{matrix}\right\} \times \begin{Bmatrix} O{+}{-} \\ B{+}{-} \end{Bmatrix}$		
	Indeterminate	$\left.\begin{matrix} A{+}{+} \\ A{+}{-} \end{matrix}\right\} \times \begin{Bmatrix} A{+}{+} \\ A{-}{+} \\ AB{+}{+} \\ AB{-}{+} \end{Bmatrix}$	$\left.\begin{matrix} A{+}{+} \\ AB{+}{+} \end{matrix}\right\} \times \begin{Bmatrix} O{+}{+} \\ B{+}{+} \end{Bmatrix}$		

TABLE 2 (continued)

PHENOTYPE	GENOTYPE	WILL BE PRODUCED BY CROSSES
$B++$	$(BM)\,(RN)$	$\left.\begin{matrix}B+-\\AB+-\end{matrix}\right\}\times\left\{\begin{matrix}O-+\\O++\\A-+\\A++\end{matrix}\right.$ $\left.\begin{matrix}B++\\AB++\end{matrix}\right\}\times\left\{\begin{matrix}O-+\\A-+\end{matrix}\right.$
	$(BN)\,(RM)$	$\left.\begin{matrix}B-+\\AB-+\end{matrix}\right\}\times\left\{\begin{matrix}O+-\\O++\\A+-\\A++\end{matrix}\right.$ $\left.\begin{matrix}B++\\AB++\end{matrix}\right\}\times\left\{\begin{matrix}O+-\\A+-\end{matrix}\right.$
	Indeterminate	$\left.\begin{matrix}B++\\B+-\end{matrix}\right\}\times\left\{\begin{matrix}B++\\B-+\\AB++\\AB-+\end{matrix}\right.$ $\left.\begin{matrix}B++\\AB++\end{matrix}\right\}\times\left\{\begin{matrix}O++\\A++\end{matrix}\right.$

TABLE 3

	s=2			s=3			s=4			s=5			s=6			s=7			s=8			s=9			s=10		
	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V
	45	2	0	3	2	1	20	3	1	16	3	2	91	4	2	119	4	3	..	..	..	118	6	3	..	..	..
	124	2	0	96	3	0	27	2	2	122	3	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	148	2	0	103	2	1	31	3	1	164	3	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	154	2	1	35	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
LANDSTEINER and	..	..	..	156	2	1	72	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
LEVINE (First Series)	..	..	..	..	..	..	104	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	..	..	..	107	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	..	..	..	110	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	..	..	..	114	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	..	..	..	146	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	..	..	..	160	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Totals		6	0		11	4		29	15		9	6		4	2		4	3					6	3			

TABLE 3 (continued)

	s=2			s=3			s=4			s=5			s=6			s=7			s=8			s=9			s=10		
	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V	FAMILY NUMBER	U	V
	6	1	1	10	2	1	21	4	0	8	3	2	4	5	1	44	5	2	51	6	2	..	..	..	108	6	4
	76	2	0	14	2	1	23	3	1	13	4	1	11	3	3	60	5	2	103	4	4	..	..	..	..	..	..
	..	..	..	25	3	0	31	3	1	17	3	2	79	4	2	81	4	3	..	..	..	..	..	..	..	..	..
	..	.	..	48	2	1	39	2	2	20	4	1	94	5	1	109	5	2	..	..	..	..	..	..	..	..	..
WIENER and	..	..	..	74	2	1	52	2	2	53	5	0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
VAISBERG	..	..	..	90	2	1	82	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	96	3	0	89	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	110	2	1	114	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	113	2	1	124	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	..	..	..	119	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Totals		3	1		22	8		25	11		19	6		17	7		19	9		10	6					6	4
	3	1	1	11	2	1	1	3	1	10	3	2	38	4	2	22	4	3	14	4	4	..	..	..	..	..	..
	42	1	1	31	2	1	27	2	2	15	3	2	..	..	..	67	5	2	..	..	..	..	..	..	..	..	..
	52	1	1	78	3	0	49	3	1	68	3	2	..	..	..	75	4	3	..	..	..	..	..	..	..	..	..
LANDSTEINER and	74	2	0	84	2	1	55	2	2	77	3	2	..	..	..	100	5	2	..	..	..	..	..	..	..	..	..
LEVINE (Second	81	1	1	111	3	0	57	4	0	89	3	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Series)	97	1	1	..	..	..	65	2	2	91	3	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	101	2	0	..	..	..	93	2	2	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	108	2	0	..	..	..	94	3	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
	110	2	0	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Totals		13	5		12	3		21	11		18	12		4	2		18	10		4	4						
Grand Totals		22	6		45	15		75	37		46	24		25	11		37	19		14	10		6	3		6	4
N=	14			30			28			14			6			8			3			1			1		
Q=	21.43 percent			25.00 percent			33.04 percent			34.29 percent			30.56 percent			33.93 percent			41.66 percent			33.33 percent			40.00 percent		

classes: linked, crossovers, and indeterminates. The indeterminates were omitted from consideration, and the number of children remaining taken equal to s, and the column the family was listed in determined. Since the crossovers and the linked types could not be differentiated, the consistent system was adopted of placing the larger number in the column headed U, and the smaller number in the column headed V. Thus, family 96 of the study of WIENER and VAISBERG is a cross of an $A+-$ father by an $A++$ mother, producing three $O+-$, four $A++$, and one $A+-$ children. According to table 1, therefore, there are no linked type children, the three $O+-$ children are crossovers, and the $A+-$ and four $A++$ children are indeterminate. This family is therefore listed under the column headed s = 3, with U = 3, V = O. The other families listed in table 3 were analyzed in a similar manner.

The columns headed U and V were then totalled, and Q, the percentage value of V, was determined. It is possible to determine the most probable value of Q for various sized families (different values of s) and for any crossover value. The results of such calculations are tabulated in table 4.

TABLE 4

Value of Q for various sized families and various crossover values.

NUMBER OF CHILDREN	CROSSOVER VALUE									
	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
s=2–3	0.0475	0.0900	0.1275	0.1600	0.1875	0.2100	0.2275	0.2400	0.2475	0.2500
s=4–5	0.0498	0.0981	0.1237	0.1856	0.2226	0.2541	0.2792	0.2976	0.3088	0.3125
s=6–7	0.0499	0.0996	0.1479	0.1938	0.2358	0.2726	0.3028	0.3252	0.3391	0.3437
s=8–9	0.0500	0.0999	0.1492	0.1971	0.2420	0.2823	0.3162	0.3418	0.3578	0.3633
s=10–11	0.0500	0.1000	0.1496	0.1985	0.2453	0.2881	0.3247	0.3530	0.3708	0.3769
s=12–13	0.0500	0.1000	0.1498	0.1992	0.2471	0.2917	0.3305	0.3610	0.3805	0.3872
s=14–15	0.0500	0.1000	0.1499	0.1996	0.2482	0.2940	0.3347	0.3671	0.3880	0.3951
s=16–17	0.0500	0.1000	0.1499	0.1998	0.2488	0.2957	0.3378	0.3718	0.3940	0.4016

NOTE: The value of Q in general, is the same for two values of s such as 2n and 2n+1.

In order to evaluate the significance of the difference between the value of Q found and its theoretically to be expected value, it is necessary to know the probable error. In table 5 the standard error of Q is given. To derive the probable error from the value given in table 5, it is necessary to multiply by the factor $0.6745/\sqrt{N}$, where N represents the number of families analyzed. The derivation of formulae for Q and its standard error are given at the end of the paper.

In table 6, the actual value of Q is compared with the values theoreti-

TABLE 5

Standard error of Q.

NUMBER OF CHILDREN	CROSSOVER VALUE									
	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
s=2	0.1466	0.1921	0.2179	0.2333	0.2420	0.2468	0.2490	0.2498	0.2500	0.2500
s=3	0.1165	0.1480	0.1639	0.1665	0.1654	0.1609	0.1552	0.1497	0.1456	0.1443
s=4	0.1211	0.1448	0.1658	0.1765	0.1802	0.1788	0.1750	0.1731	0.1668	0.1653
s=5	0.0964	0.1284	0.1452	0.1545	0.1548	0.1467	0.1388	0.1305	0.1239	0.1218
s=6	0.0871	0.1210	0.1408	0.1514	0.1548	0.1527	0.1467	0.1411	0.1337	0.1313
s=7	0.0803	0.1118	0.1294	0.1417	0.1397	0.1353	0.1301	0.1172	0.1094	0.1069
s=8	0.0769	0.1068	0.1241	0.1383	0.1392	0.1392	0.1313	0.1225	0.1151	0.1118
s=9	0.0705	0.0995	0.1156	0.1262	0.1295	0.1271	0.1188	0.1080	0.0989	0.0955
s=10	0.0689	0.0937	0.1119	0.1225	0.1273	0.1268	0.1206	0.1112	0.1092	0.0993

TABLE 6

CROSSOVER VALUE	s=2			s=3			s=4		
	Q	DEV.	P.E.	Q	DEV.	P.E.	Q	DEV.	P.E.
0.50	25.00	−3.57	4.51	25.00	0.00	2.18	31.25	+1.79	2.12
0.45	24.75	−3.32	4.46	24.75	+0.25	2.20	30.88	+2.16	2.13
0.40	24.00	−2.57	4.33	24.00	+1.00	2.26	29.76	+3.28	2.21
0.35	22.75	−1.32	4.10	22.75	+2.25	2.34	27.92	+5.12	2.23
0.30	21.00	+0.43	3.79	21.00	+4.00	2.43	25.41	+7.63	2.28
Actual	21.43			25.00			33.04		
CROSSOVER VALUE	s=5			s=6			s=7		
	Q	DEV.	P.E.	Q	DEV.	P.E.	Q	DEV.	P.E.
0.50	31.25	+3.04	2.18	34.37	−3.81	3.53	34.37	−0.46	2.55
0.45	30.88	+3.41	2.23	33.91	−3.35	3.68	33.91	+0.02	2.61
0.40	29.76	+4.53	2.35	32.52	−1.96	3.80	32.52	+1.41	2.80
0.35	27.92	+6.37	2.50	30.28	+0.28	4.04	30.28	+3.65	3.10
0.30	25.41	+8.88	2.64	27.26	+3.30	4.20	27.26	+6.67	3.22
Actual	34.29			30.56			33.93		
CROSSOVER VALUE	s=8			s=9			s=10		
	Q	DEV.	P.E.	Q	DEV.	P.E.	Q	DEV.	P.E.
0.50	36.33	+5.33	4.35	36.33	−3.00	6.44	37.69	+2.31	6.70
0.45	35.78	+5.88	4.48	35.78	−2.45	6.67	37.08	+2.92	7.36
0.40	34.18	+7.48	4.77	34.18	−0.85	7.28	35.30	+4.70	7.50
0.35	31.62	+10.04	5.11	31.62	+1.71	8.01	32.49	+7.51	8.13
0.30	28.23	+13.43	5.42	28.23	+5.10	8.57	28.81	+11.19	8.55
Actual	41.66			33.33			40.00		

TABLE 7

	ACTUAL		CROSSOVER FREQUENCY									
			0.50		0.45		0.40		0.35		0.30	
	TOTAL NUMBER OF CHILDREN	v	v	$(P.E._v)^2$	v	$(P.E._v)^2$	v	$(P.E._v)^2$	v	$(P.E._v)^2$	v	$(P.E._v)^2$
s=2	28	6	7.00	1.59	6.93	1.56	6.72	1.46	6.27	1.32	5.88	1.12
s=3	60	15	15.00	1.72	14.85	1.74	14.40	1.79	13.56	1.96	12.60	2.13
s=4	112	37	35.00	5.62	34.59	5.71	33.33	6.15	31.27	6.25	28.46	6.50
s=5	70	24	21.88	2.34	21.62	2.43	20.83	2.69	19.54	3.06	17.79	3.34
s=6	36	11	12.37	1.61	12.21	1.74	11.71	1.88	10.90	2.10	9.81	2.28
s=7	56	19	19.25	2.04	18.89	2.13	18.20	2.47	16.96	3.03	15.27	3.24
s=8	24	10	8.72	1.08	8.59	1.16	8.20	1.30	7.59	1.51	6.78	1.69
s=9	9	3	3.27	0.34	3.22	0.36	3.08	0.42	2.85	0.51	2.53	0.59
s=10	10	4	3.77	0.45	3.71	0.54	3.53	0.56	3.25	0.66	2.88	0.74
Totals	405	129	126.26	16.79	124.71	17.37	120.00	18.72	112.28	20.40	102.00	21.63
Deviation			2.74		4.29		9.00		16.72		27.00	
Probable error				4.09		4.17		4.33		4.51		4.65
Dev./P.E.			0.67		1.03		2.08		3.68		5.81	

cally to be expected for various crossover frequencies. For an assumed crossover value of 50 percent (assumption of independence), the deviation was found to be less than the probable error for s = 2, 3, 4, 7, 9, 10, and less than twice the probable error for s = 5, 6, 8. The results, therefore, do not contradict the assumption that *M* and *N* are inherited independently of *A* and *B*. For an assumed crossover frequency of 30 percent, on the other hand, the deviation was found to be more than three times the probable error for s = 3, 4; and more than twice the probable error for s = 7, 8. A linkage intensity as great as 70 percent can therefore certainly be excluded. Furthermore, for an assumed crossover frequency of 35 percent, the deviation is almost two and a half times the probable error for s = 3, 4, so that the existence of a linkage intensity even as small as 65 percent is rendered unlikely.

It is possible to bring our conclusions within narrower limits by combining the results for the various sized families. This is done in table 7. The desired result was accomplished by converting the percentage expectancies (Q) into the corresponding absolute values (V) for each sized family, and then obtaining the total absolute expectancies for V for the various crossover values. These results were compared with the total absolute value of V actually found. The absolute value of the probable error was also calculated. It was found that the deviation was more than three times the probable error for an assumed crossover value of 35 percent, so that a linkage intensity of 65 percent can now certainly be excluded. The actual value of V found agrees best with the expectancy for a crossover value of 50 percent (equivalent to the assumption of independence). Considering the fact that there are twenty-four pairs of chromosomes, so that *a priori* the likelihood of linkage is very small, our results indicate that the agglutinogens *M* and *N* are most probably inherited independently of *A* and *B*.

DERIVATION OF FORMULA FOR Q

If p represents the true linkage intensity and q the crossover frequency (so that p + q = 1) and s equals the number of "determinate" children, then the probability that μ of these children be linked types and ν be crossovers is given by:

$$w_{\mu,\nu} = \frac{s!}{\mu!\nu!} p^{\mu} q^{\nu}.$$

Then,

$$Q = \Sigma \frac{\mu}{s} \frac{s!}{\mu!\nu!} (p^{\mu} q^{\nu} + p^{\nu} q^{\mu}), \text{ where } \mu \text{ is less than } \nu.$$

This may be reduced to:

$$Q = pq + (pq)^2 + 2(pq)^3 + 5(pq)^4 + 14(pq)^5 + 42(pq)^6 + 132(pq)^7 + 429(pq)^8 + \cdots . \quad (1)$$

To determine the value of Q from this formula, s/2 terms are taken if s is even, and (s−1)/2 terms are taken if s is odd. Thus, if s=7, and p=0.50 (so that pq=0.25), Q=(0.25)+(0.25)²+2(0.25)³ or Q=0.34375.

The remaining values of Q given in table 4 were calculated in a similar manner from formula (1).

DERIVATION OF FORMULA FOR STANDARD ERROR OF Q

The standard error of Q may now be derived as follows:

Let

$$\frac{s!}{\mu!\nu!}p^\mu q^\nu = A_\mu$$

Then,

$$Q = \Sigma\frac{\mu}{s}(A_\mu + A_{(s-\mu)}).$$

Let A_μ' be the actual frequency of families with μ children of linked types and $(s-\mu)$ children of crossover types found in a study of N families of s children each.

Then,

$$A_\mu' = A_\mu + x_\mu$$

$$\sqrt{N}\,\sigma_{A_\mu} = \sqrt{A_\mu(1 - A_\mu)} = \sqrt{\Sigma x_\mu^2}.$$

Similarly, if Q′ equal the actual value found and Q the theoretically to be expected value,

$$Q' - Q = \Sigma\frac{\mu}{s}(x_\mu + x_{(s-\mu)}) = x_Q$$

$$N \cdot \sigma_Q^2 = \Sigma x_Q^2 = \Sigma\frac{1}{s^2}[\Sigma\mu(x_\mu + x_{(s-\mu)})]^2$$

Since,

$$\Sigma x_\mu x_{(s-\mu)} = 0$$

$$N\sigma_Q^2 = \Sigma\frac{1}{s^2}[\Sigma\mu^2(x_\mu^2 + x_{(s-\mu)}^2) + \Sigma 2\mu\nu(x_\mu + x_{(s-\mu)})(x_\nu + x_{(s-\nu)})]$$

$$\Sigma x_\mu^2 = N\sigma_{A_\mu}^2 = A_\mu(1 - A_\mu)$$

$$\Sigma x_\mu x_\nu = N r_{\mu\nu}\sigma_\mu\sigma_\nu .$$

Since $A_1, A_2, \cdots, A_{s-1}$ form a Bernoulli system,

$$r_{\mu\nu} = -\sqrt{\frac{A_\mu A_\nu}{(1-A_\mu)(1-A_\nu)}}$$

so that

$$\Sigma x_\mu x_\nu = -A_\mu A_\nu$$

$$N\sigma_Q^2 = \frac{1}{s^2}\{\Sigma\mu^2[A_\mu(1-A_\mu) + A_{(s-\mu)}(1-A_{(s-\mu)})] - 2\Sigma\mu\nu(A_\mu + A_{(s-\mu)})(A_\nu + A_{(s-\nu)})\}$$

$$= \frac{1}{s^2}\{\Sigma\mu^2(A_\mu + A_{(s-\mu)}) - [\Sigma\mu^2(A_\mu^2 + A_{(s-\mu)}^2) + \Sigma 2\mu\nu(A_\mu + A_{(s-\mu)})(A_\nu + A_{(s-\nu)})]\}$$

$$= \frac{1}{s^2}\{\Sigma\mu^2(A_\mu + A_{(s-\mu)}) - [\Sigma\mu(A_\mu + A_{(s-\mu)})]^2\}$$

$$\sqrt{N}\,\sigma_Q = \frac{1}{s}\sqrt{\Sigma\mu^2(A_\mu + A_{(s-\mu)}) - [\Sigma\mu(A_\mu + A_{(s-\mu)})]^2}.$$

Since,

$$\Sigma\frac{\mu}{s}(A_\mu + A_{(s-\mu)}) = Q$$

and since it can be shown that

$$\Sigma\frac{\mu^2}{s^2}(A_\mu + A_{(s-\mu)}) = Q - \frac{s-1}{s}pq,$$

then,

$$\sqrt{N}\,\sigma_Q = \sqrt{Q - \frac{s-1}{s}pq - Q^2}.$$

Thus, if $s=5$ and $p=q=\frac{1}{2}$

$$Q = 0.3125 \text{ (from table 4)}$$

$$Q^2 = 0.09766$$

$$\frac{s-1}{s}pq = 0.20$$

$$\sqrt{N}\sigma_Q = \sqrt{0.01484} = 0.1218.$$

In making our calculations we have not taken into account the effect of selection of families sometimes necessary because a doubly heterozygous parent can often only be recognized from the phenotypes of the children. Thus three kinds of cases may be distinguished:

(1) When studying linkage of the LANDSTEINER blood groups and the agglutinogens *M* and *N*, if one parent is of type *AB*++, we know immediately that this parent is doubly heterozygous, as the blood group *AB* and type *M*+*N*+ are both heterozygous.

(2) If the parent in question is of type *A*++, however, we cannot tell if he is doubly heterozygous unless at least one child is of group *O*.

(3) When studying linkage of blood groups and eye color, for example, if the parent in question is of group *A* and has brown eyes, we can only be certain he is doubly heterozygous if at least one child is of group *O* and at least one child has blue eyes.

In the second and third cases, therefore, there will be selection of families. The values of Q and its standard error given in tables 4 and 5 apply only to the former two matings. For the third kind of mating a correction is necessary. Thus for the first series of LANDSTEINER and LEVINE a correction is necessary for families 16, 20, 27, 31, 35, 45, 72, 93 and 96, which were examined for agglutinogen *M* but not for agglutinogen *N*. This correction is small and does not materially affect the final results, however.

SUMMARY

A method of measuring linkage that may be applied in human genetics is presented. The method of application is illustrated by an analysis of data on the heredity of the agglutinogens *A*, *B*, *M*, and *N* in order to determine whether or not linkage exists between the agglutinogens *M* and *N* and the agglutinogens *A* and *B*.

Considering the fact that there are twenty-four pairs of chromosomes so that *a priori* the probability of linkage between any two factors is small, and also considering the fact that the results of the analysis fit best with the assumption of independence of *M* and *N* from *A* and *B*, it becomes most probable that *M* and *N* are inherited independently of *A* and *B*. Our findings therefore confirm those of LANDSTEINER and LEVINE, and of BERNSTEIN.

The method used depends upon the elimination of the necessity for the determination of the genotype of the heterozygous plant. This is accomplished by dividing the children into two main classes, linked and crossover types. Since the two classes cannot be differentiated, the system was

adopted of calling the larger class U and the smaller class V. The total value of V for all the families examined can then be compared with the expectancies for various assumed linkage intensities. BERNSTEIN attained a similar result by taking the product of the two classes, thus eliminating the necessity for determining the genotype of the heterozygous parent.

LITERATURE CITED

BERNSTEIN, F., 1931 Zur Grundlegung der Chromosomentheorie der Vererbung beim Menschen mit besonderer Berücksichtung der Blutgruppen. Z. indukt. Abstamm.-u. Vererb-Lehre. **57:** 113–138.

DAVENPORT, C. B., 1927 Heredity of human eye color. Bibl. genet. **3:** 443–463.

1930 Sex linkage in man. Genetics **15:** 401–444.

HAECKER, V. 1925 Pluripotenerscheinungen, Synthetische Beitrage zur Vererbungs- und Abstammunglehre. Fischer: Jena.

KUBANYI, A., 1931 Weitere Untersuchungen über die Beziehung zwischen Hemophilieverebung und Blutgruppencharakter, Klin. Wchnschr. **10:** 597–601.

LANDSTEINER, K., and LEVINE, P., 1928 On individual differences in human blood. J. Exp. Med. **47:**757–775.

1928 On the inheritance of agglutinogens of human blood demonstrable by immune agglutinins. J. Exp. Med. **48:** 731–748.

LEVINE, P., 1926 Atopic hypersensitiveness. J. Immunol. **11:** 283–297.

MADLENER, M., 1928 Eine Bluter-Familie, Arch. Rass.-u. Ges. Biol. **20(4):** 390–394.

MORGAN, T. H., 1926 The theory of the gene. 358 pp. Yale Univ. Press: New Haven, Conn.

SNYDER, L., 1929 Blood grouping and its relation to clinical and legal medicine. Williams and Wilkins: Baltimore, Md.

1931 Linkage in man. Eugenical News **16:** 177–179.

WIENER, A. S. and VAISBERG, M., 1931 Heredity of the agglutinogens *M* and *N* of LANDSTEINER and LEVINE. J. Immunol. **20:** 371–388.

FIRST-CHROMOSOME GENES IN THE TOMATO[1]

E. W. LINDSTROM
Iowa State College, Ames, Iowa

Received November 19, 1931

TABLE OF CONTENTS

	Page
Introduction	351
Linear order of first-chromosome genes	352
Fruit-size relations with the first-chromosome genes	354
Manifold effects of the *Ss* genes	355
Summary	357
Literature cited	357

Introduction

The linear order of the four known genes on the first chromosome of the tomato has never been critically established. The genes concerned are as follows: *Dd* (tall-dwarf growth habit), *Pp* (smooth-pubescent epidermis), *Oo* (oblate-ovate, major fruit shape) and *Ss* (simple-compound inflorescence).

Several years ago I succeeded in developing the quadruple recessive form essential for the requisite backcross. This was inbred for two generations and then crossed reciprocally with the quadruple dominant, in this case the commercial variety Marglobe. Reciprocal backcrosses of this F_1 to the quadruple recessive type were grown, and these furnish the major portion of data for this report. This backcross may be designated as follows:

$$F_1(ddppooss \times DDPPOOSS) \times ddppooss.$$

The dwarf, peach, ovate, compound-cluster parent was small-fruited while the Marglobe parent was large-fruited.

The determination of the fruit-shape genes, *Oo*, was obtained from measurements of the equatorial and blossom-stem end (polar) diameters of ten fruits from each plant, a shape index being calculated therefrom. This is necessary because these *Oo* genes are not the only oblate or ovate shape factors in the tomato. They are one of the major pairs however.

Fruit size as measured by fruit weight was also obtained from ten fruits per plant.

Complete data were secured from a greenhouse planting of 139 backcross plants, and this was repeated in a field planting of 150 plants.

[1] Paper No. 41., Department of Genetics, Iowa State College.

LINEAR ORDER OF FIRST-CHROMOSOME GENES

The combined results of the greenhouse and field plantings are arranged in table 1 so as to show the data from the reciprocal backcrosses separately.

TABLE 1

Backcross data involving four pairs of linked genes from the cross F_1(DPOS·dpos)×ddppooss and reciprocal.

COMBINATIONS	F_1 AS FEMALE	F_1 AS MALE	TOTAL	PERCENT
DPOS	79	46	125	
dpos	52	33	85	72.7
DPos	7	7	14	
dpOS	15	5	20	11.8
DPOs	13	3	16	
dpoS	5	8	13	10.0
Dpos	2	2	4	
dPOS	4	0	4	2.8
DPoS	4	2	6	
dpOs	0	0	0	2.1
DpoS	1	0	1	
dPOs	1	0	1	0.7
Total	183	106	289	100.1
Number of crossovers	52	27	..	..
Percent	28.4	25.5	..	..

Three sets of single and two of double crossovers were noted, but no triple crossing over occurred. The linear order of the genes proved to be *D–P–O–S*. In this experiment the relative map distances between the genes were as follows: *D* 3.5 *P* 13.9 *O* 12.8 *S*. Additional data to be recorded later will give better measures of linkage values. These, coupled with MACARTHUR'S (1928) data, afford verification of the linear order and relative map distances noted above.

Double crossing over in the adjoining *P–O–S* regions occurred 2.1 percent of the time where 1.8 percent would be expected with no interference. This gives a coincidence value of more than one, indicating no interference in these relatively long regions. The same general situation was found in respect to double crossing over in the more widely separated regions *D–P* and *O–S*.

The data in table 1 show that in this particular cross the total amount of crossing over in microsporogenesis was 25.5 percent and in megasporogenesis 28.4 percent. The difference of 2.9 percent is not significant in terms of its probable error. Approximately equal crossing over in the two sexes of the tomato has been reported earlier by MACARTHUR (1928) and LINDSTROM (1927).

TABLE 2

Additional linkage data on first-chromosome genes. F_2 generations.

PEDIGREE NUMBER-PARENTS	*XY*	*Xy*	*xY*	*xy*	PERCENT CROSSING OVER	PUBLISHED* PERCENTS	BACKCROSS DATA FROM TABLE 1
684—*DP*×*dp*	112	7	3	38			
619—(*XY*×*xy*)	89	1	0	28			
691—	132	2	7	38			
872—	141	1	4	46			
1087—	234	5	7	44			
Total	708	16	21	194	4.0	3.4	3.5
684—*DO*×*do*	106	13	11	30			
872—	134	8	10	40			
Total	240	21	21	70	12.6	11.4 11.0	17.4
684—*PO*×*po*	106	9	11	34			
872—	137	8	7	40			
Total	243	17	18	74	10.3	8.6 10.9	13.9
684—*Os*×*oS*	76	41	42	1	14.4	20.0	12.8
684—*Ds*×*dS*	80	39	38	3			
691—	101	33	41	4			
Total	181	72	79	7	29.7	28.3	24.6
684—*Ps*×*pS*	75	40	43	2			
691—	105	34	37	3			
Total	180	74	80	5	25.4	24.5	22.5

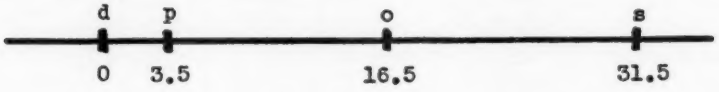
* Combined data from MACARTHUR (1928) and LINDSTROM (1925, 1926, 1927, 1928).

Additional linkage data involving the *D–P–O–S* genes are available in table 2 where a comparison of the linkage values from several sources has

been arranged. While there are the inevitable variations in crossing-over percentages, the data on the whole are in good accord.

The combined data afford a more accurate estimate of the linkage intensities of these first-chromosome genes. It has not seemed advisable to average mathematically all the results since many of the earlier determinations were based on F_2 repulsion data which are often notoriously unreliable.

For practical, present-day purposes the map of the first chromosome may be given approximately as follows:



FRUIT-SIZE RELATIONS WITH THE FIRST-CHROMOSOME GENES

Earlier reports (LINDSTROM 1926, 1927, 1928) have predicated the existence of a major fruit-size gene on this first chromosome of the tomato. The backcrosses already referred to bear on this situation directly since the quadruple-recessive parent was small fruited and the quadruple-dominant P_1 (Marglobe) was large fruited. The backcross to the small-fruited type does not afford the most critical evidence on size inheritance because of the partial dominance of the smaller size. But since no large-fruited, ovate, dwarf, peach, compound-cluster types are in existence, the present arrangement is the only possible test.

In table 3 are given the frequency distributions of fruit size as measured by fruit weight in the greenhouse and field plantings of the backcross generations, involving the parental, single and double crossover combinations of the four linked genes.

A comparison of the fruit weights in the first two columns (*DPOS* and *dpos*) shows a very evident association of the larger fruits with the dominant *DPOS* genes as well as of the smaller fruits with the *dpos* genes. The most critical evidence is afforded in the weight distributions of the single crossover *DPos* and *dpOS* (third and fourth columns) combinations. Here it is plainly evident, in both plantings, that fruit size is associated with something in the *O–S* end of the chromosome. The next two columns (five and six) which are differentiated on the *Ss* genes exhibit no marked differences in fruit weight. Accordingly fruit size (or weight) is apparently affected by something very near the *Oo* genes. That this something is a size gene cannot be proved by these data, although experiments already

reported (LINDSTROM 1928) and some not yet concluded indicate that such is actually the case.

TABLE 3

Distribution of fruit weights in relation to the four pairs of linked genes on the first chromosome. P_1—small size ddppooss×large size DDPPOOSS.

WEIGHT IN GRAMS	*DPOS*	*dpos*	*DPos*	*dpOS*	*DPOs*	*dpoS*	*Dpos*	*dPOS*	*DPoS*	*DpoS*	*dPOs*
					GREENHOUSE PLANTING						
23	..	2	2	..	..	..	..	..	..	..	..
26	1	3	1	..	..	..	..	..	..	..	..
29	3	10	2	..	..	3	..	..	1	..	..
32	5	7	4	..	..	4	..	..	1	..	..
35	8	8	1	1	3	3	1	1	1	..	..
38	11	5	..	3	4	1	0	0	1	..	..
41	5	2	..	0	4	0	1	0	..	..	..
44	8	2	..	1	..	0	..	1	..	..	..
47	1	..	..	2	..	1	..	..	..	..	..
50	0	..	..	0	..	..	..	..	..	..	..
53	5	..	..	2	..	..	..	..	..	..	..
56	2	..	..	1	..	..	..	..	..	..	..
Total	49	39	10	10	11	12	2	2	4	..	..
					FIELD PLANTING						
25	3	5	2	..	1	..	..	..	..	..	..
30	6	5	2	..	1	..	..	..	1	..	..
35	17	9	..	..	1	..	..	..	0	..	..
40	16	14	..	..	0	1	2	..	0	..	1
45	17	9	..	2	0	..	..	..	0	..	..
50	10	2	..	1	1	..	..	2	1	..	..
55	6	1	..	3	1	..	..	..	..	1	..
60	0	..	..	1	..	..	..	..	..	..	..
65	0	..	..	0	..	..	..	..	..	..	..
70	1	..	..	3	..	..	..	..	..	..	..
Total	76	45	4	10	5	1	2	2	2	1	1

MANIFOLD EFFECTS OF THE *Ss* GENES

The *Ss* genes have been named for their most obvious expression which is concerned with the inflorescence, simple or compound. The source of the recessive, compound-cluster *s* gene in the material reported above was the commercial variety Grape Cluster. It was observed that there were two other important effects of this gene. First, the number of flower clusters formed on the compound type was always less than is formed on the ordinary varieties of tomatoes. Whereas the usual number of nodes, or

number of leaves between the various flower clusters, on the main stem of the standard type is three, the number on compound-cluster types is usually four or five.

Second, the compound-cluster variety, Grape Cluster, carries noticeably beaked fruits. In some cases the blossom end of the fruit is sharply beaked, the beak extending as far as 4–5 millimeters beyond the normal end of the fruit.

It is of interest to determine whether or not these developments, thus associated with the *s* gene, are invariably correlated with this gene. Quantitative data for the number of nodes between flower clusters are available in the backcross generations of the greenhouse planting shown in table 4. Here the number of leaves between each of the first three flower clusters is averaged and arranged in relation to the linked *OoSs* genes.

TABLE 4

Relation of genes OoSs and the number of leaves between each of the first three flower clusters.

AVERAGE NUMBER OF LEAVES BETWEEN FRUIT CLUSTERS	*OS*	*Os*	*oS*	*os*	*S*	*s*	*O*	*o*
3	41	1	10	3	51	4	42	13
4	19	6	4	33	23	39	25	37
5	1	4	2	15	3	19	5	17
Total	61	11	16	51	77	62	72	67

Evidently the *Ss* genes are more closely correlated with the number of leaves than are the *Oo* genes. The average number of leaves between flower clusters is 3.4 for the *S* type and 4.2 for the *s* type, a significant difference. The data indicate a rather close relation between the *Ss* genes and number of nodes, although some variation exists. It must be recalled that all the simple-cluster individuals in this experiment were heterozygous *Ss*, and since the *S* gene does not show complete dominance, although it approaches it closely, there probably is some diluting effect on the other manifestations of this gene.

The degree of beaking in relation to these same *OoSs* genes is given in table 5.

Here again it is the *Ss* genes that show the closest association with the beaking of the fruit. Such evidence makes it clear that these genes have a very pronounced and deep-seated influence on both plant and fruit characters. That this influence traces to the single genes *Ss* and not to other genes linked with them is not proved by the foregoing data. Only general

experience with the compound-cluster gene in other crosses leads me to suggest the hypothesis of manifold effect.

TABLE 5

Relation of OoSs genes and beaking of fruits.

	OS	*Os*	*oS*	*os*	*S*	*s*	*O*	*o*
Non-beaked	53	0	10	0	63	0	53	10
Slightly beaked	8	2	6	3	14	5	10	9
Medium beaked	0	5	0	32	0	37	5	32
Strongly beaked	0	4	0	16	0	20	4	16
Total	61	11	16	51	77	62	72	67

SUMMARY

The linear order of the four known genes on the first chromosome of the tomato was established by a four-point, backcross test as *D–P–O–S*.

Evidence is presented of the probable location of an important fruit-size gene on the first chromosome. This gene is located very near the *Oo* fruit-shape genes.

The manifold effects of the *Ss* inflorescence genes are described and measured. They influence not only the type of the flower clusters but also the number of clusters and the beaking of the fruit.

LITERATURE CITED

LINDSTROM, E. W., 1925 Inheritance in tomatoes. Genetics **10**: 305–317.

1926 Linked inheritance in tomatoes. Iowa State Coll. J. Sci. **1**: 3–13.

1927 The inheritance of ovate and related shapes of tomato fruits. J. Agric. Res. **34**: 961–985.

1928 Linkage of size, shape and color genes in Lycopersicum. Proceedings of the 5th International Congress, Supplementband of the Z. indukt. Abstamm.-u. VererbLehre. **2**: 1031–1057.

MACARTHUR, J. W., 1926 Linkage studies with the tomato. Genetics **11**: 387–405.

1928 Linkage studies with the tomato. II. Three linkage groups. Genetics **13**: 410–420.

THE NATURE AND EXTENT OF HETERO-FERTILIZATION IN MAIZE

GEORGE F. SPRAGUE

Bureau of Plant Industry, United States Department of Agriculture, Washington, District of Columbia

Received November 20, 1931

TABLE OF CONTENTS

	PAGE
INTRODUCTION	358
Gametogenesis and fertilization	358
Inheritance of scutellum color	359
The phenomenon of hetero-fertilization	359
Genetic tests of the nature of hetero-fertilization	361
Persistence of the four megaspores	362
Mutation	362
Non-disjunction	363
Sperms from two pollen grains	366
The extent of hetero-fertilization	367
SUMMARY	367
LITERATURE CITED	368

INTRODUCTION

The term hetero-fertilization has been applied (SPRAGUE 1929) to the process resulting in those exceptional cases in which the embryo and endosperm differ genetically. It has been suggested that these exceptions may occur because (a) the egg and polar nuclei are of different genetic constitution and fuse with identical sperms or, conversely, (b) the egg and polar nuclei are of the same genotype but at syngamy fuse with sperms having unlike genotypes. These conditions in maize may be brought about in various ways. The persistence and functioning of the four megaspores, mutations, non-disjunction of one or more chromosome pairs when the generative nucleus divides to form the sperms, or the functioning of unlike sperms from two pollen grains might result in hetero-fertilized kernels. A brief resumé of the cytological happenings prior to and during syngamy will facilitate the explanation of this phenomenon.

GAMETOGENESIS AND FERTILIZATION

Gametogenesis and fertilization in maize have been described in detail by MILLER (1919) and WEATHERWAX (1919). MILLER concluded that all four of the megaspores persisted as in the Lilium type of embryo sac. WEATHERWAX, previously of the same opinion, states in his 1919 paper

that "only the chalazal one of the four megaspore potentialities persists." The embryo sac at the time of fertilization shows the egg decidedly alveolar in appearance. The synergids are considerably longer than the egg and may show signs of disintegration before fertilization occurs. The antipodals have undergone very rapid division from the eight cell stage and may present the appearance of a definite tissue. The polar nuclei are imbedded in a strand of cytoplasm connecting the egg and antipodals. They retain their identity until fertilization takes place.

In the anther, after formation of microspores, the microspore nucleus divides equationally giving rise to a vegetative and a generative nucleus. Before anthesis the generative nucleus divides again to produce two crescent shaped sperms. Pollen grains falling on the silk germinate, sending out pollen tubes which grow down the styles. Only a single tube reaches the embryo sac, which it penetrates, usually causing the destruction of one of the synergids. The two sperms are discharged into the embryo sac where one fuses with the egg to give rise to the scutellum and the embryo proper. The second sperm fuses with the two polar nuclei. This final triple fusion product gives rise to the endosperm of which the aleurone layer is a part. Genetic investigations so far reported for maize are in conformity with these findings.

INHERITANCE OF SCUTELLUM COLOR

Purple and red scutellum color has been shown to be rather complex in its mode of inheritance (SPRAGUE 1932). Before purple or red color can develop in the scutellum, the fundamental aleurone factors A, C, R, and i must be present. The P_r p_r factor pair which differentiates purple and red aleurone has a similar effect on scutellum color. The development of scutellum color is also dependent upon at least five other factors which interact in the following manner: S_1 must always be present in a dominant condition. Any two of the three factors S_2, S_3, and S_4, must likewise be present in a dominant condition. Finally, the factor pair s_5 must be present in a homozygous recessive condition. Such a system of interaction permits the existence of homozygous colored scutellum strains of four different genotypes. In several cases in this paper the genotypic identity of a particular true breeding strain has not been determined. The factor complex resulting in colored scutellum in these instances is designated S_x, or, if the strain in question has colorless scutellum, s_x.

THE PHENOMENON OF HETERO-FERTILIZATION

Seeds with white aleurone normally have no purple or red scutellum color and, when selfed, either produced only white aleurone kernels or

kernels segregating in ratios characteristic of the action of an inhibitory factor. In 1925 and 1926, however, a few kernels were found with colorless aleurone and colored scutellums (figure 1). When these kernels were planted and the progeny selfed, the resulting ears showed segregation for both scutellum and aleurone color (figure 2). This was unexpected since the parent seeds had colorless aleurone and the subsequent segregation for aleurone color did not indicate the presence of an inhibitory factor. Thus, the kernels with colored scutellums and colorless aleurone were peculiar in two respects. Colored scutellums were present with colorless aleurone and the progeny of these colorless aleurone seeds segregated for aleurone color in subsequent generations characteristically like that previ-

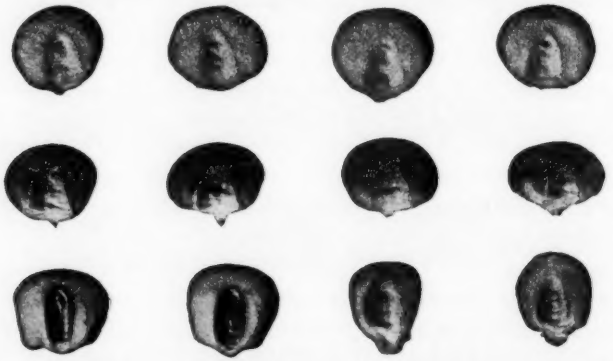


FIGURE 1.—Normal kernels with colorless aleurone, colorless scutellums (top row), colored aleurone, colored scutellums (middle row), and hetero-fertilized kernels with colorless aleurone and colored scutellums (lower row).

ously reported only from hybrid colored seeds. The aberrant seeds just described have been called hetero-fertilized since the embryo and endosperm are of different phenotypes.

It should perhaps be emphasized that the phenomenon of hetero-fertilization is not confined to progenies segregating for aleurone and scutellum coloration. Considerable evidence is available which indicates that hetero-fertilization occurs occasionally in all corn. Hetero-fertilization has been observed by Professor R. A. EMERSON of CORNELL UNIVERSITY and Mr. F. D. RICHEY of the UNITED STATES DEPARTMENT OF AGRICULTURE. In neither case were there large numbers of such seeds, and because their material was not favorable for a study of this condition, their observations were never reported.

The relation between aleurone and scutellum factors, however, provides an ideal situation for detecting this phenomenon. Advantage has been taken of the ease of identifying hetero-fertilized kernels having colorless aleurone and colored scutellums to investigate their occurrence. Kernels having colored aleurone and colorless scutellums likewise may be hetero-fertilized, but, as they cannot be identified without obtaining selfed

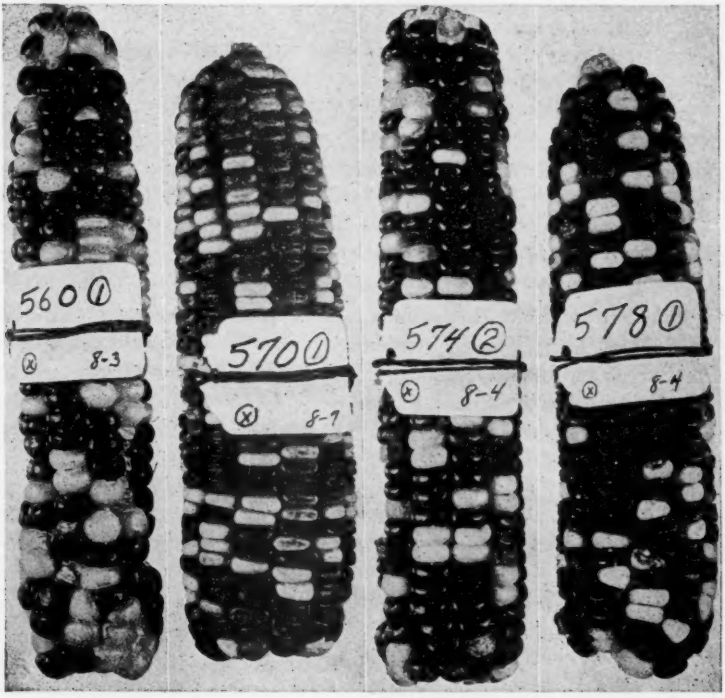


FIGURE 2.—Selfed ears obtained from the hetero-fertilized kernels shown in figure 1.

progeny, they have not been used in this study further than to establish the fact that they occur approximately as frequently as their complementary type.

GENETIC TESTS OF THE NATURE OF HETERO-FERTILIZATION

Genetic tests have been made of three of the four hypotheses mentioned as possible causes of hetero-fertilization. The results of these tests indicate

that at least the majority of all hetero-fertilized seeds are due to the functioning of genotypically unlike sperms from two pollen grains.

Among the selfed progeny of the first hetero-fertilized seeds studied was one ear which had a high percentage of these aberrant seeds. A high hetero-fertilized stock from this ear has been used in the tests reported here.

Persistence of the four megaspores

Cytologic and genetic evidence so far reported is in agreement in indicating that three of the four megaspores die. Since hetero-fertilization ordinarily does not occur with a high frequency, and since the number of young embryo sacs observed by cytologists must necessarily have been limited, there remains the possibility that hetero-fertilization is due to the persistence and functioning of the megaspore tetrad. The production of hetero-fertilized seeds due to the persistence of the four megaspores in an embryo sac of either the Lilium or Smilacina type would depend on the genotypes of the spores and their subsequent rôle in the formation of the egg and fusion nuclei. If the four spores persisted and the eight nuclei functioned at random, plants from a high hetero-fertilization stock heterozygous for the essential scutellum and aleurone factors would be expected to produce some hetero-fertilized seeds when pollinated with a multiple recessive stock. Representative data from such crosses are presented in table 1. No hetero-fertilized seeds have been obtained in these tests.

TABLE 1

Normal and hetero-fertilized kernels on ears of the cross $AACcRr\ S_x \times AcR\ s_x$.*

PEDIGREE NUMBER	COLORED ALEURONE	COLORLESS ALEURONE	HETERO-FERTILIZATION
632–1×493–2	138	146	0
–2×493–2	142	149	0
–3×493–2	146	152	0
–4×493–2	102	95	0
–5×493–2	147	143	0
–6×493–2	88	85	0
Total	763	770	0

* S_x denotes the complex required for colored scutellums, s_x that for colorless scutellums, but with the particular combination of factors in question unidentified.

Mutation

The relative mutation rates of the aleurone factors *A*, *C* and *R* have been studied by STADLER (1930). Of the three genes, *R* was shown to mutate most frequently, one mutation occurring for every 2500 gametes tested.

Mutations of the scutellum factors have not been observed but probably are of no more frequent occurrence than those of the aleurone series. Since hetero-fertilized seeds appear with approximately equal frequency in progenies segregating for each of the three aleurone factors, and since hetero-fertilization occurs about thirty times as frequently as mutation at the *R* locus, mutation of an aleurone factor or factors does not offer a satisfactory explanation of this phenomenon.

Non-disjunction

The records for aleurone color in 65 selfed progenies grown from hetero-fertilized seeds (colorless aleurone and colored scutellum) are presented in table 2. The segregations serve as a test of non-disjunction as the causal agency of hetero-fertilization. To effect hetero-fertilization, non-disjunction of one of the chromosome pairs carrying aleurone factors must take place at the equational division of the generative nucleus. For illustration we may assume that the II chromosome is involved and that the generative nucleus in question carries *R*. Non-disjunction would result in sperms of two types n+1 (*RR*) and n−1 (*O*). To obtain a hetero-fertilized seed the n+1 sperm must fuse with an egg having the factor *r*. The embryo would then be *RRr* and the endosperm *rro*. The selfed progeny of such a seed should segregate in a ratio of 35 colored to 1 colorless aleurone individual if there were no selection against n+1 gametes.

In a study of extra chromosome individuals derived from a triploid, McClintock (1929) has shown that n+1 ♂ gametes seldom function in competition. In addition, there appeared to be some selection against n+1 ♀ gametes since equal numbers of 2n and 2n+1 plants were not obtained from backcrosses. The data presented indicated that approximately 70 percent of the n+1 ♀ gametes functioned. With the complete elimination of n+1 ♂ gametes and a 30 percent elimination of n+1 ♀ gametes, the theoretical ratio approximates 14 to 1 for the duplex condition and 2 to 1 for the simplex.

In table 2 among the ears showing a single factor segregation for aleurone color, there are four ears whose Dev./P.E. exceeds 3, due to deficiencies in the colorless aleurone class. The observed ratio for these four ears taken as a group is 4.43 to 1. While these segregations are undoubtedly aberrant, it seems clear that the deviations obtained cannot be considered as random fluctuations from the expected 14 to 1. In the same group are two progenies whose ratios approximate the expected 2 to 1 for the simplex trisome. The observed ratio for colored to colorless aleurone in these two progenies (570–1 and 574–2) is 2.13 to 1.

TABLE 2

Numbers of kernels with aleurone and scutellum color for 65 selfed ears grown from hetero-fertilized seed (colorless aleurone, colored scutellum).

PEDIGREE	ALEURONE			SCUTELLUM				HETERO-FERTILIZED KERNELS
	COLORED	COLORLESS	DEV./P.E.	COLORED	COLORLESS	RATIO	DEV./P.E.	
				3:1 ratio for aleurone color				
559–1	260	82	0.65	252	8	15:1	3.14	1
561–1	59	22	0.67	46	13	3:1	0.78	0
–2	187	72	1.54	81	106	27:37	0.46	2
–3	173	61	0.56	125	48	3:1	1.24	2
567–1	222	79	0.74	168	54	3:1	0.34	4
570–1	290	136	4.89	216	74	3:1	0.30	0
571–1	122	44	0.66	100	22	3:1	2.63	0
574–1	194	79	2.23	180	14	15:1	0.83	0
–2	236	111	4.46	232	4	15:1	4.28	2
575–1	236	77	0.24	178	58	3:1	0.22	0
577–1	180	57	0.50	96	84	9:7	1.17	1
578–1	271	91	0.09	238	33	54:10	2.32	0
579–4	247	78	0.62	231	16	15:1	0.22	1
580–1	158	57	0.76	127	31	3:1	2.32	0
582–5	176	62	0.55	73	103	27:37	0.28	0
583–2	207	62	1.10	148	59	3:1	1.73	1
–3	206	65	0.57	156	50	3:1	0.36	0
585–1	123	25	3.38	56	67	27:37	1.09	1
586–1	300	96	0.52	300	0	..	..	3
–2	142	36	2.18	122	20	54:10	0.75	3
587–1	237	82	0.43	227	10	15:1	1.92	0
–2	121	53	2.47	111	10	15:1	1.36	0
588–1	266	73	2.18	266	0	..	..	1
590–1	158	52	0.12	151	7	15:1	1.40	5
–3	125	31	2.19	125	0	..	..	1
–4	182	43	3.03	183	0	..	..	3
593–1	107	22	3.09	107	0	..	..	1
–2	177	62	0.50	177	0	..	..	2
594–1	84	33	1.19	80	4	15:1	0.83	0
–2	206	51	2.83	206	0	..	..	0
596–1	200	48	3.04	119	81	9:7	1.37	0
597–2	334	125	1.64	250	84	3:1	0.09	0
598–1	75	26	0.26	67	8	54:10	1.62	0
–2	184	54	1.22	155	29	54:10	0.08	1
–3	32	13	0.89	32	0	..	..	0
–4	22	4	1.68	21	1	15:1	0.49	0
718–1	59	23	0.95	59	0	..	..	1
Total 3:1	6559	2187	0.01					36

TABLE 2 (continued)

PEDIGREE	ALEURONE			SCUTELLUM				HETERO-FERTILIZED KERNELS
	COLORED	COLORLESS	DEV./P.E.	COLORED	COLORLESS	RATIO	DEV./P.E.	
				9:7 ratio for aleurone color				
341–2	45	32	0.57	24	21	9:7	0.59	0
342–1	275	182	2.51	218	57	3:1	2.43	0
558–1	132	130	2.84	122	10	15:1	0.93	2
–2	119	95	0.28	113	6	15:1	0.80	8
560–1	137	105	0.17	73	64	9:7	1.04	0
–3	121	109	1.65	60	61	27:37	2.44	0
–4	106	74	1.06	56	50	9:7	1.05	1
563–2	37	25	0.81	30	7	3:1	1.26	0
–3	128	109	1.03	92	36	3:1	1.21	2
564–1	127	90	1.00	99	28	3:1	1.14	0
565–2	146	126	1.27	108	38	3:1	0.42	0
568–1	137	115	0.89	106	31	3:1	0.95	0
569–4	101	86	0.91	33	68	27:37	2.85	0
571–2	224	182	0.65	116	108	9:7	2.00	0
579–1	27	22	0.25	27	0	..	..	1
–2	206	165	0.42	193	13	15:1	0.06	1
581–1	99	77	0.00	99	0	..	..	29
582–1	147	112	0.24	106	41	3:1	1.20	0
–2	128	101	0.16	118	10	15:1	1.08	0
–3	88	72	0.47	56	32	9:7	2.07	0
–4	137	97	1.05	107	30	3:1	1.24	0
597–1	108	89	0.60	49	59	27:37	0.99	0
Total 9:7	2775	2195	0.14					44
				27:37 ratios for aleurone color				
569–1	122	180	0.93	62	60	9:7	1.79	0
–6	30	31	1.64	14	16	27:37	0.76	0
579–3	21	23	1.10	20	1	15:1	0.41	0
581–3	173	200	2.44	158	15	15:1	1.95	23
595–1	133	187	0.34	133	0	..	..	2
718–2	21	28	0.15	15	6	3:1	0.56	14
Total 27:37	500	649	0.43					39

Among the ears exhibiting a 9:7 or 27:37 segregation for aleurone coloration, the deviations of the individual ears and of the groups are well within the limits of random sampling. These results indicate that nondisjunction may occasionally result in hetero-fertilized seeds but is inadequate to account for the phenomenon in general.

Sperms from two pollen grains

Selfed ears were selected from the high hetero-fertilization stock which were homozygous for the factors necessary for colored scutellum with the exception of the aleurone factor *R* which was segregating. Colorless aleurone, colorless scutellum segregates from these progenies were grown and pollinated with a pollen mixture composed of approximately equal parts by volume of its own (*r*) and sib (*R*) pollen. If hetero-fertilization is due to the functioning of unlike sperms from two pollen grains, an occasional seed with colored scutellum and colorless aleurone is to be expected. Some of the results from mixed pollinations of this type are presented in table 3. Hetero-fertilized seeds are seen to occur very frequently. These results indicate that the usual cause of hetero-fertilization is the functioning of sperms from two pollen grains of unlike genotypes.

TABLE 3

Numbers of normal and hetero-fertilized kernels on ears resulting from the cross $ACrS_s \times \begin{cases} ACRS_s \\ + \\ ACrS_s \end{cases}$

PEDIGREE NUMBER	TOTAL KERNELS		HETERO-FERTILIZED KERNELS
	R	*r*	
993–6 × 993+ 994	10	10	1
–10 × 993+ 994	8	21	8
6786–6 ×6787+6790	75	60	29
7830–2 ×7830+7829	107	60	18
–2½×7830+7829	53	72	3
–3 ×7830+7833	38	88	12
7834–1 ×7834+7833	34	83	28
Totals	325	394	99

Is there some protective mechanism in maize which ordinarily limits the functioning male nuclei to those derived from a single pollen tube? In animal eggs this protective mechanism is a heavy membrane which develops around the egg after fertilization. No such structure has been reported in the higher plants and even if one were universally present it would not interfere with the process of hetero-fertilization. The usual protective mechanism in plants must be sought in the embryo sac and its enveloping tissues or in the style. No evidence is available at present which may distinguish between these possibilities.

THE EXTENT OF HETERO-FERTILIZATION

Hetero-fertilization seems to be widespread, and occurs rather frequently. Since such kernels cannot be detected in most material without recourse to their selfed progeny, unusual behavior due to this phenomenon has doubtless frequently been ascribed to a faulty classification of the parent seed. The frequencies of hetero-fertilized kernels with colorless aleurone, and colored scutellums among the colorless aleurone class in progenies segregating for aleurone and scutellum color were 1 in 50 and 1 in 81 for all such progenies grown in 1928 and 1929, respectively. Only the colorless aleurone class was used in these calculations since it is the only one in which the phenomenon can be detected by direct observation.

The above results suggest that hetero-fertilization occurred more frequently in 1928 than in 1929. This difference, however, is due to the inclusion of excess progenies from the high hetero-fertilization stock in 1928. When these are omitted from the averages, the frequency becomes 1 in 76 which is in excellent agreement with the 1929 data. Within the high hetero-fertilization stock the phenomenon occurs much more frequently as shown by the data in table 3. Among the 394 colorless aleurone kernels there occurred 99 cases of hetero-fertilization or 1 such case for every 4 seeds obtained.

Among progenies segregating in a 3 to 1 ratio for aleurone color, only a small proportion of the possible hetero-fertilizations will produce kernels with colorless aleurone and colored scutellums. The theoretical proportion of such kernels increases with the number of aleurone factor pairs heterozygous. In table 2, hetero-fertilized kernels with colorless aleurone and colored scutellums occurred with a frequency of 1 in 60 for the 3 to 1 group, 1 in 49 for the 9 to 7 group, and 1 in 17 for the 27 to 37 group. Using the frequency found for the 3 to 1 group as a basis for calculation and assuming the factors necessary for colored scutellums to be present equally, the expected frequencies are 1 in 60, 1 in 34, and 1 in 20, respectively. The agreement is fair. Apparently hetero-fertilized kernels occur approximately in the proportions expected from the frequencies of the possible unlike sperm combinations which may cause them.

SUMMARY

An aberrant condition is described for maize in which the endosperm and embryo are of different phenotypes. The process by which this is accomplished is called hetero-fertilization.

Genetic tests indicate that hetero-fertilization is due to the fusion of the egg and of the polar nuclei with sperms of unlike genotypes.

Non-disjunction may play a minor rôle in producing these genotypically different sperms.

Results from mixed pollinations indicate that the genotypic differences are usually due to the participation in the fertilization process of sperms from more than one pollen grain.

Hetero-fertilization occurs with a frequency of approximately 1 seed in 80 in normal cultures and 1 in 4 in the high hetero-fertilization stock.

LITERATURE CITED

McCLINTOCK, B., 1929 A cytological and genetical study of triploid maize. Genetics **14**: 180–222.

MILLER, E. C., 1919 Development of the pistillate spikelet and fertilization in *Zea mays*. J. Agric. Res. **18**: 255–265.

SPRAGUE, G. F., 1929 Hetero-fertilization in maize. Science **69**: 526–527.

1932 The inheritance of scutellum colors in maize. U. S. Dept. Agric. Tech. Bull. 292.

STADLER, L. J., 1930 The frequency of mutation of specific genes in maize. Anat. Rec. **47**: 381.

WEATHERWAX, PAUL, 1919 Gametogenesis and fecundation in *Zea mays* as the basis of xenia and heredity in the endosperm. Bull. Torrey Bot. Cl. **46**: 73–90.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, of cytology, taxonomy, embryology, physiology, biometry, or mathematics, if of sufficient importance and of such a character as to be of primary interest to the geneticist. For the present, the length of manuscripts will be limited to twenty-five printed pages (about twelve thousand words) except by special vote of the Editorial Board. Tabular matter in excess of one-fourth of the manuscript can not be printed, unless of particular importance, but will be kept on file for reference on request provided two copies are furnished by the author. Excess pages will be printed if paid for by the author (about six dollars a page).

Manuscripts are printed, ordinarily, in the order of their receipt. They may be printed out of turn provided the entire cost is paid by the author. Such material will be added to the current number and will not delay the publication of any other article.

Contributors are requested to use care in the preparation of manuscripts. Carbon copies can not be considered. All references to literature should cite the name of the author, followed by the year of publication, the papers so referred to being collected into a list of "Literature cited" at the end of the article. In this list care should be taken to give the titles in full, and to indicate accurately, in Arabic numerals, the volume number, the first and last pages, and the date of publication of each paper if published in a periodical, and the number of pages, place and date of publication, and the name of publisher, of each independent publication. The arrangement of this list should be alphabetical by author and chronological under each author. Titles of publications are abbreviated according to the *World list of scientific periodicals*, Oxford University Press, New York, 1925.

Factor symbols should be separated so that they can be properly identified and underlined for italics. All but the first letter of each symbol will be printed in subscript or superscript. Most typewriters do not distinguish between the letter l and the figure 1 or the hyphen and the dash. Such distinctions should be made wherever there is a possibility of confusion.

Names of species are printed in italics but genera alone are not and should be marked accordingly.

Footnotes should be avoided wherever possible. Usually, they can be enclosed in parentheses and inserted after the sentences to which they apply. If used in the text they should be numbered consecutively in a single series and designated by Arabic superscript numerals. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in the tables.

Illustrations should be referred to as figures wherever possible. Plates are reserved for illustrations that require paper inserts or for collections of small figures that can not be designated conveniently as separate figures. Text figures should be clearly identified but not numbered in the illustration. All figures and plates are reduced to a maximum of 4⅜ inches in width and 6 inches in height.

Legends for figures and plates should be typewritten separately from the illustrations for the reason that the type is set by the printer and the illustrations are made by the engraver. Mistakes are likely to occur if the separation is not made by the author.

Galley proofs and, whenever there is time, page proofs will be sent. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript. Ordinarily page proofs can not be sent out of the country. Both proofs must be returned promptly and no extensive change may be made in page proofs, which is not compensated for within the same paragraph, or in an adjacent paragraph on the same page. Changes from copy will be charged against the author.

GENETICS pays for one-half the cost of reprints up to 100 copies. Additional copies may be secured at actual cost of manufacture provided these are ordered when the corrected galley proofs are returned.

Manuscripts and all editorial correspondence should be addressed to the Editor of GENETICS, P.O. Box 1106, New Haven, Connecticut.

GENETICS, MAY, 1932

TABLE OF CONTENTS

PAGE

RILEY, H. P., Self-sterility in shepherd's purse..... 231

DAWSON, W. M., Inheritance of wildness and tameness in mice.... 296

EAST, E. M., Further observations on *Lythrum salicaria*..... 327

WIENER, A. S., Method of measuring linkage in human genetics; with special reference to blood groups..... 335

LINDSTROM, E. W., First-chromosome genes in the tomato..... 351

SPRAGUE, G. F., The nature and extent of hetero-fertilization in maize..... 358

SIXTH INTERNATIONAL CONGRESS OF GENETICS

Out of the necessary indefiniteness inherent in the present period of economic uncertainty have come a number of facts concerning the SIXTH INTERNATIONAL CONGRESS OF GENETICS which are definitely encouraging and of interest to all American geneticists. Some months ago the Council was worried concerning memberships and possible attendance at the CONGRESS. This is no longer the case, five hundred memberships having been obtained. This insures a successful and representative gathering at Ithaca. In spite of necessary and frequent changes in transportation arrangements, it is certain that a number of the more outstanding European geneticists will also be in attendance. Economic conditions make an accurate prediction of an exact number impossible at this time. There will, however, undoubtedly be a sufficient representation of foreign geneticists to form the nucleus of an excellent invitation program. The Committee on Exhibits has under way plans for the most comprehensive and extensive demonstration of material which has been gathered at any meeting. It is certain that this feature, including as it will much living plant and animal material, will be an outstanding feature of the CONGRESS. It is confidently hoped and expected that the work of every genetics laboratory in the United States and Canada will be exhibited and that material will also be received from many European and foreign laboratories. The Local Committee reports that the Administration of CORNELL UNIVERSITY has quoted rates in residential halls for a period of four to seven days, of $1.75 per day. Private rooming houses, adjoining the campus, quote rates of from $1.00 to $1.50 per day, depending on the facilities offered and length of occupancy. It is planned to publish, during the early summer, a more detail survey of facilities and rates for the information of members. The replies to this will serve as a guide in making final arrangements. The question of membership in the CONGRESS for more than one in a family has been considered by the Council. It has been decided that after one member of a family has joined the CONGRESS as an active member (either full or student membership) additional members of the family may join at $2.00 each. In such cases only one copy of the *Proceedings* will be issued to each family. From time to time, as items of interest arise, bulletins will be issued and notices published in *Science*. Geneticists are urged to communicate with the secretary or treasurer of the CONGRESS concerning membership if they have not already done so. If membership has already been obtained and literature is not being received, the secretary will appreciate word to that effect.

[This number of GENETICS (17: 231–368) was issued May 2, 1932]